E14 and S7B Clinical and Nonclinical Evaluation of QT/QTc Interval Prolongation and Proarrythmic Potential—Questions and Answers

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FOREWORD

The International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use (ICH) has the mission of achieving greater regulatory harmonization worldwide to ensure that safe, effective, and high-quality medicines are developed, registered, and maintained in the most resource-efficient manner. By harmonizing the regulatory expectations in regions around the world, ICH guidelines have substantially reduced duplicative clinical studies, prevented unnecessary animal studies, standardized safety reporting and marketing application submissions, and contributed to many other improvements in the quality of global drug development and manufacturing and the products available to patients.

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International Council for Harmonisation (ICH)

E14 and S7B Clinical and Nonclinical Evaluation of QT/QTc Interval Prolongation and Proarrhythmic Potential--Questions and Answers

Draft Questions & Answers (Q&As)

Endorsed by the ICH Assembly under *Step 2a* and endorsed by the ICH Regulatory Members of the Assembly under *Step 2b* on 27 August 2020
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Note: This draft guidance contains:

- Revisions proposed to sections 5.1 and 6.1 of the current Q&As for ICH E14. Other sections are not proposed for revision. Subsequently, when finalized, the revised sections 5.1 and 6.1 will be integrated with the other current Q&As for ICH E14;
- New Q&As for ICH S7B.

References

ICH E14	The Clinical Evaluation of QT/QTc Interval Prolongation and Proarrhythmic Potential for Non-Antiarrhythmic Drugs	May 2005
ICH E14	Questions & Answers (R3)	Dec 2015
ICH S7B	Nonclinical Evaluation of the Potential for Delayed Ventricular Repolarization (QT Interval Prolongation) by Human Pharmaceuticals	May 2005

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5. Use of Concentration Response Modeling of QTc Data

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5.1		The ICH E14 Guideline states (in Section 3.2.3, page 10) that analysis of the relationship between drug concentration and QT/QTc interval changes is under active investigation. Has this investigation yielded a reasonable approach to concentration-response modeling during drug development? How can assessment of the concentration-response relationship guide the interpretation of QTc data?	Concentration-response analysis, in which all relevant data across all doses are used to characterize the potential for a drug to influence QTc, can serve as an alternative to the by-time-point analysis or intersection-union test as the primary basis for decisions to classify the risk of a drug. In either case, this result is an important component of the totality of evidence assessment of the risk of QT prolongation. The overall assessment of risk of QT prolongation includes nonclinical data, the time course of QT prolongation, the magnitude of QT prolongation, categorical analyses of outliers, and certain adverse events in patients that can signal potential proarrhythmic effects. There are many different types of models for the analysis of concentration-response data, including descriptive pharmacodynamic (PD) models (e.g., linear or Emax models), or empirical models that link pharmacokinetic (PK) models (dose-concentration-response) with PD models. It is recognized that concentration-response analyses of the same data using models with different underlying assumptions can generate discordant results. Therefore, it is important that the modeling methods and assumptions, criteria for model selection, rationale for model components, and potential for pooling of data across studies be specified prior to analysis to limit bias. Prospective specification of model characteristics (e.g., structural model, objective criteria, goodness of fit) based on knowledge of the pharmacology is recommended whenever possible. On occasion, the QT effect is not a direct function of plasma concentration. For example, drugs that cause QT prolongation as a result of changes in protein synthesis or trafficking or drugs with accumulation into myocardial tissues might demonstrate hysteresis. Testing for model assumptions, hysteresis (a plot of data by-time point and a hysteresis loop plot), and goodness of fit should be documented. Concentration-response analysis can be challenging when more than one molecular entity-multiple drugs or parent plus metabolites-contributes to the QTc effect. **Important considerations**

			Concentration-response data need not come from a dedicated QT study, nor even a single study, but there are several new and important considerations. 1. Data can be acquired from first-in-human studies, multiple-ascending dose studies, or other studies provided that the concentrations achieved are well above the exposure at the maximum therapeutic dose at steady-state, and reflect high exposure scenario situations such as drug-drug and drug-food interactions, organ dysfunction, and/or genetically impaired metabolism. 2. Efficient concentration-response analysis using data acquired in studies with other purposes requires as much quality control as is needed for a dedicated study. This includes robust, high-quality electrocardiogram (ECG) recording and analysis sufficient to support a valid assay for ECG intervals (see ICH E14 and Q&A 1). 3. If there is an intention to pool data from multiple studies, it is important to test for heterogeneity. Pooling of studies that were not planned for this purpose can produce bias. This potential should be critically discussed in the analysis plan. 4. A separate positive control would not be necessary if either of the following conditions is met: a) There are data characterizing the response at a sufficiently high multiple of the clinically relevant exposure (see ICH E14 Section 2.2.2); or b) If the maximum therapeutic exposure has been fully covered in the clinical ECG assessment (e.g., concentrations representative of the maximum recommended dose at steady-state in situations of intrinsic and/or extrinsic factors that increase bioavailability), but sufficiently high multiples cannot be obtained (e.g., for reasons of safety, tolerability, saturating absorption), then a nonclinical integrated risk assessment that includes the hERG assay, an *in vivo* QT assay, and any follow up studies can be used as supplementary evidence. See ICH S7B Q&A 1.1 for details; in summary, the nonclinical studies should include (1) a hERG safety margin higher than the safety margins computed under the same experimental protocol for a series of drugs known to cause torsade de pointes (TdP) and (2) no QTc

			prolongation in an *in vivo* assay of sufficient sensitivity conducted at exposures of parent compound and human-specific major metabolites that exceed clinical exposures. **Decision-making** Both the intersection-union test and the concentration-response analysis can estimate the maximum effect of a drug treatment on the QTc interval, but they are not used to test the same hypothesis. As mentioned above, inspection of the time course of QT prolongation is important. However, hypothesis testing based on a by-time point analysis (intersection-union test or point estimate and confidence intervals) is inappropriate in studies designed for a concentration-response analysis, if not powered to assess the magnitude of QT prolongation for each time point. When using a concentration-response analysis as the primary basis for decisions to classify the risk of a drug, the upper bound of the two-sided 90% confidence interval for the QTc effect of a drug treatment as estimated by exposure-response analysis should be <10 ms at the highest clinically relevant exposure to conclude that an expanded ECG safety evaluation during later stages of drug development is not needed. (See ICH E14, Section 2.2.4 and Q&A 7). **Other uses** In addition to serving as the basis for regulatory decision-making, concentration-response analysis has established its utility in several settings enumerated below. <u>Providing insight into regimens not studied directly</u> An understanding of the concentration-response relationship can help predict the QT effects of doses, dosing regimens, routes of administration, or formulations that were not studied directly. Interpolation within the range of concentrations studied is more reliable than extrapolation above the range. <u>Predicting QTc effects of intrinsic and extrinsic factors that affect pharmacokinetics</u> Understanding the concentration-response relationship can help predict the effects of intrinsic (e.g., cytochrome P450 isoenzyme status) or extrinsic (e.g., drug-drug PK interactions) factors, possibly affecting inclusion criteria or dosing adjustments in later phase studies.

6. Special Cases

#	Date of Approval	Questions	Answers
6.1		The ICH E14 Guideline states that in certain cases a conventional thorough QT study might not be feasible. In such cases what other methods should be used for evaluation of QT/QTc and proarrhythmic potential?	An integrated nonclinical and clinical QT/QTc risk assessment can be particularly valuable under scenarios where a placebo-controlled comparison is not possible; safety considerations preclude administering supratherapeutic doses to obtain high clinical exposures and/or safety or tolerability prohibit the use of the product in healthy participants. The design elements that include placebo and healthy participant dosing assist in decreasing variability, but their absence does not preclude interpretation. The integrated nonclinical and clinical QT/QTc risk assessment should include: 1. The hERG assay, an *in vivo* QT assay, and any follow-up nonclinical studies, especially those selected to overcome the challenges encountered in the clinical studies (see ICH S7B Q&As 1.1 and 1.2); and 2. Alternative QT clinical study designs incorporating ECG assessments with as many of the usual "thorough QT/QTc" design features as possible (see ICH E14 Section 2.2 and Q&A 5.1). In situations where it is not possible to evaluate the QT/QTc effects at higher exposures than are anticipated with the recommended therapeutic dose, it is particularly important that the nonclinical *in vivo* studies are conducted at exposures exceeding the clinical therapeutic exposures. An integrated QT/QTc risk assessment can also be particularly valuable for drugs with confounding heart rate effects (i.e., >20 bpm) that could impact accurate determination of the QTc. Advanced methodologies for controlling or correcting for heart rate changes in the nonclinical *in vivo* studies and/or conducting QTc assessments in patients with the disease might be informative in this situation. If tolerance to the chronotropic effect develops with repeat dosing, upward titration regimens can sometimes be employed to

avoid or minimize the confounding effects of drug-induced heart rate changes on the QTc assessment.

Decision-Making

A totality of evidence argument based on the results of an integrated nonclinical and clinical QT/QTc assessment could be made at the time of marketing application. To support a drug as having low likelihood of proarrhythmic effects due to delayed repolarization, the assessment should demonstrate the following:

1. The nonclinical studies, following best practice considerations for *in vitro* studies (see ICH S7B Q&A 2) and *in vivo* studies (see ICH S7B Q&A 3), show low risk which includes (1) a hERG safety margin higher than the safety margins computed under the same experimental protocol for a series of drugs known to cause TdP; and (2) no QTc prolongation in an *in vivo* assay of sufficient power to detect a QTc prolongation effect of a magnitude similar to dedicated clinical QT studies and at exposures of parent compound and human-specific major metabolites that exceed clinical exposures (see ICH S7B Q&A 1.1 for details).
2. The high-quality ECG data (see ICH E14 and E14 Q&A 1) collected in the alternative QT clinical assessment do not suggest QT prolongation, generally defined as ΔQTc greater than 10 ms, as computed by the concentration-response analysis (see E14 Q&A 5.1 for details) or the intersection-union test. The strength of the clinical ECG data depends on the upper bound of the two-sided 90% confidence interval around the mean ΔQTc estimate. If applicable, there should be no notable imbalances between treatment/dose arms in the proportion of subjects exceeding outlier thresholds.
3. A cardiovascular safety database that does not suggest increased rate of adverse events that signal potential for proarrhythmic effects (ICH E14 Section 4).

The ICH E14/S7B Implementation Working Group is seeking input *via* public comment on how to define the lack of clinically relevant QT prolongation in the context of the specific #2 criteria above when #1 and #3 would also be met.

			If nonclinical studies do not show low risk (or are not performed), there is reluctance to draw conclusions of lack of an effect in an absence of a positive control; however, if the upper bound of the two-sided 90% confidence interval around the estimated maximal effect on ΔQTc is less than 10 ms, the treatment is unlikely to have an actual mean effect as large as 20 ms.

**Note that E14 Q&As 6.2 and 6.3 are not being revised*

New S7B Q&As

1. Integrated Risk Assessment

#	Date of Approval	Questions	Answers
1.1		What is the general strategy for use of nonclinical information as part of an integrated risk assessment for delayed ventricular repolarization and torsade de pointes that can inform the design of clinical investigations and interpretation of their results?	The ICH S7B guideline describes a nonclinical strategy for assessing risk of delayed ventricular repolarization and QT interval prolongation (Section 2.3). A mechanistic understanding of the development of torsade de pointes (TdP) and the emergence of new types of assays have made it possible to obtain more information to assess TdP risk from nonclinical assays. The *in vitro* IKr/hERG assay and *in vivo* QT assay as well as optional follow-up studies, as described by the ICH S7B guideline, are conducted for hazard identification and risk assessment relevant to delayed ventricular repolarization. It is generally accepted that drugs (note that the word "drug(s)" in the Q&As is used interchangeably with word "pharmaceutical(s)" in ICH S7B) that delay ventricular repolarization may have increased risk of TdP. These nonclinical assays should be performed prior to human testing to support the planning and interpretation of First-in-Human clinical studies. Nonclinical investigations can also contribute to an integrated risk assessment for TdP in later stages of development when clinical data are available. The following are points to consider when using *in vitro* IKr/hERG data and *in vivo* QT data in combination with clinical QT data as part of an integrated risk assessment for situations described in ICH E14 Q&As 5.1 & 6.1. 1. To predict whether or not the hERG block poses a risk of interfering with ventricular repolarization or TdP, evaluation of the hERG safety margin based on results of a best practice assay (see S7B Q&As 1.2 and 2.1) is recommended. Factors that would influence the interpretation of the safety margin include the ability of the drug to block other cardiac ion channels,

			the potential for large excursions in clinical exposure due to intrinsic or extrinsic factors and the contributions of metabolites that inhibit the hERG channel. 2. In the *in vivo* study, the effects on the QTc interval should be assessed at exposures that cover the anticipated high clinical exposure scenario. The adequacy of exposure to any major human-specific metabolites should be determined (see ICH S7A Sections 2.3.3.2 & 2.6, and S7B Q&A 3.5). In addition, if the assay is to be used as part of an integrated clinical and nonclinical risk assessment for situations where a conventional thorough QT study is not feasible as described in ICH E14 Q&A 6.1, the *in vivo* study should have sufficient power to detect a QTc prolongation effect of a magnitude similar to dedicated clinical QT studies. A drug with low TdP risk would be expected to have (1) a hERG safety margin higher than the safety margins computed under the same experimental protocol for a series of drugs known to cause TdP; and (2) no QTc prolongation in an *in vivo* assay of sufficient sensitivity conducted at exposures of parent compound and human-specific major metabolites that exceed clinical exposures. If these results are used to support an integrated clinical and nonclinical risk assessment strategy as described in ICH E14 Q&As 5.1 & 6.1, no additional nonclinical studies are needed, except when there are factors that can confound or limit the interpretation of the nonclinical studies, such as metabolites and heart rate changes. Under those situations, follow-up studies as described by ICH S7B (Section 2.3.5) can be performed to address these specific issues. If the hERG assay and/or the *in vivo* QT study suggest an effect at clinical exposures, the drug has a risk of interfering with ventricular repolarization. Under this scenario, the drug's TdP risk could be affected by various other factors, such as blocking of additional repolarization currents (e.g., IKs), blocking of inward currents (e.g., sodium and L-type calcium currents), effects on the trafficking of ion channel proteins from cytoplasmic sites to the surface membrane, metabolites with ion channel activities, and non-ion channel mediated QT prolongation.

			Follow-up studies (ICH S7B Section 2.3.5) could be performed to further explore the mechanisms and assess the TdP risk. If applicable, best practice considerations should be followed for assessment of additional ion channel currents (S7B Q&A 2.1), *in vitro* cardiomyocyte assays (S7B Q&As 2.2–2.4), or *in vivo* studies (S7B Q&As 3.1–3.5). An appropriately qualified proarrhythmia risk prediction model (see S7B Q&As 4.1–4.3) could be used according to its context of use to assess the possibility of TdP in humans. The assessment of TdP risk using these follow-up studies, although optional, can be used together with other relevant nonclinical and clinical information to contribute to the design of subsequent clinical investigations and interpretation of their results.
1.2		What is the recommended method to compute the hERG safety margin?	A drug's potency for hERG block, usually calculated as half-inhibitory concentration (IC_{50}), can be normalized to the drug's estimated clinically relevant exposures (e.g., free C_{max} at steady state) in patients to calculate the safety margin. As more information is obtained during the clinical development, the estimated values of clinical exposures can be refined. When estimating hERG block potency, it is recommended to use standardized procedures and to consider the principles described in S7B Q&A 2.1. The free drug exposure is computed based on the drug's total plasma concentration and the fraction of protein binding. Because of uncertainties in the protein binding measurements, the unbound (free) fraction in plasma should be set to 1% if experimentally determined to be < 1%. If protein binding values cannot be accurately assessed (e.g., questionable validation of the bioanalytical method, deviations from best practices, and/or concentration-dependency of binding characteristics) or if tissue levels are likely to exceed free plasma concentrations, safety margins should be calculated for both steady-state free and total C_{max}. To assess whether the hERG block poses a risk of delaying ventricular repolarization or TdP, the resulting safety margin should be compared to the range of safety margins computed under the same experimental protocol for a series of drugs that have known clinical TdP risk and cover diverse electrophysiological

			properties. If there is an intention to comment on safety margins, the aforementioned data for the reference compounds should be supplied in or appended to the submitted study report. Appropriate statistical methods should be applied to quantify experimental variability and calculate uncertainty of safety margin as confidence/credible intervals.

2. Best Practice Considerations for *In vitro* Studies

#	Date of Approval	Questions	Answers
2.1		What are some "best practice" considerations when evaluating drug potency on affecting cardiac ionic currents using patch clamp method and overexpression cell lines?	As outlined in ICH S7B, the *in vitro* IKr/hERG assay plays a critical role in assessing the risk for delayed repolarization and QT interval prolongation prior to first administration in humans. Nonclinical investigations can also contribute to an integrated risk assessment in later stages of development when clinical QT data are available. The following "best practice" considerations are intended to apply when sponsors are using nonclinical data to support interpretation of clinical QT data in specific scenarios as described in S7B Q&As 1.1 & 1.2 and ICH E14 Q&As 5.1 & 6.1. It is not the intent of these Q&As to make specific recommendations for a sponsor's screening activities or for all IKr/hERG assays to support first administration in humans. Several experimental factors are known to influence the potency of drug effects on cardiac ionic currents. These include the voltage protocols used to evoke specific ionic currents, experimental conditions (such as recording temperature, composition of solutions, manual vs. automated assay systems), data acceptance criteria, and data analysis methods employed. Some recommended best practices are therefore provided to enhance reproducibility of *in vitro* results and the translation to clinical findings. These recommendations are generalizable to voltage clamp experiments characterizing potency of drug inhibition (or potentiation) of cardiac currents.

			1. Recording temperature: The effects of some drugs are temperature-sensitive and there is currently no method to predict which molecules exhibit temperature-dependent effects or the magnitude of these effects. Thus, patch clamp experiments on cells overexpressing ion channels should be performed at near physiological temperature (35–37°C). 2. Voltage protocol: The voltage protocols used to evoke ionic currents should approximate the appropriate elements of a ventricular action potential and be repeated at physiologic intervals to ensure examination and capture of frequency-dependent effects of the test drug. The voltage protocol should include steps that enable monitoring of cell health and consistent electrophysiological recordings throughout the experiment (i.e., estimation of input and series resistance across time). If high seal resistance is achieved, holding current and input resistance (i.e., measures of passive membrane properties at rest) can be used as indicators of cell health and experimental stability. After application of the test drug and if recording quality remains acceptable, a saturating concentration of a selective blocker should be applied to cells to determine residual background current. If prominent, background current should be factored into potency determinations. 3. Recording quality: Seal resistance should be high enough so that the leak conductance at all voltages specified by the voltage protocol and series resistance do not compromise voltage control. The extent of series resistance compensation applied to optimize voltage control should be noted. Stability of the ionic current should be demonstrated with baseline recordings (prior to drug application) of sufficient duration to characterize drug-independent changes (such as current run-down). The time course of drug effects should be monitored until steady state effect is obtained, and each cell can be exposed to one or more drug concentrations as long as cell health and recording quality remain stable. 4. Primary endpoint measures: The primary derived endpoints are inhibitory concentration such as the IC_{50} value (reported in both

			micromolar and ng/mL units) and Hill coefficient. If 50% current inhibition could not be achieved, a justification of the highest concentration tested should be provided together with the relation of this concentration to therapeutic free and total drug levels. Where necessary, to isolate the current-of-interest, the background current remaining after a high concentration of selective blocker application should be subtracted. If current inhibition with a selective blocker could not be achieved, leak current can be calculated and subtracted from the current traces. This approach assumes that only the current-of-interest is voltage-dependent, hence evidence and justification should be provided on why it was used. 5. Data summary: Inhibition at each drug concentration for each cell should be provided, along with the mean values of IC_{50} and Hill coefficient (and appropriate measures of data variability). To demonstrate recording quality, the study report should also contain time-course plots of current amplitude, input resistance, and holding current for individual cells in control condition followed by drug application. If time-dependent changes such as current run-up or run-down in baseline condition were corrected for drug inhibition estimation, the correction method applied should be described. 6. Concentration verification: The concentration of compound to which the cells were exposed should be verified by applying a validated analytical method to the solution collected from the cell chamber. Both nominal and measured concentrations should be reported. If the nominal and measured concentrations differ significantly from each other, measured concentrations should be used to construct the concentration-response relationship to estimate IC_{50} and Hill coefficient. 7. Positive and negative controls: The effects of a positive control at two or more concentrations spanning 20–80% block should be used to demonstrate assay sensitivity. If positive control data fall outside the range of expected values, then the study is inconclusive, and the data

			should not be used to support the purposes outlined in ICH E14 Q&As 5.1 and 6.1. Vehicle (negative) controls should be included in the experiments. The vehicle should include all non-compound materials in the test article solution such as solubilizing agents and preservatives.
2.2		What are the relevant endpoints of an informative *in vitro* human cardiomyocyte repolarization follow-up study?	As outlined in ICH S7B, follow-up studies (Section 2.3.5) can include *in vitro* ventricular repolarization assays. Follow-up studies are not performed with all submissions and are often designed to address specific issues. Since implementation of ICH S7B, new technologies have become available, including assays with human induced pluripotent stem cell derived cardiomyocytes (hiPSC-CMs). S7B Q&As 2.2–2.4 outline best practice considerations when *in vitro* cardiomyocyte assays are performed as follow-up studies. Drug-induced changes in the intracellular or extracellular action potential waveforms recorded from hiPSC-CM preparations and acutely isolated adult human ventricular myocytes reflect the integrated effect on multiple ionic currents, exchangers, and carriers. Changes in cellular repolarization recognized as markers of ventricular proarrhythmia include delayed and abnormal repolarization (manifest as early afterdepolarizations, triggered activity or irregular beating), and should be noted. Changes in myocyte contractions or calcium transients could have a role in further clarifying a drug's electrophysiological effects subsequently manifest as altered contractile responses (e.g., premature contractions linked to triggered electrical activity, altered calcium transients linked to calcium homeostasis). Evidence should be provided that such effects are not due to direct drug actions on electromechanical coupling or contractility.
2.3		What elements of the test system need to be considered for an *in vitro* human cardiomyocyte repolarization assay?	It is important to describe the biological preparation and technology platform that define baseline electrophysiological characteristics and drug responses. • Biological preparation: The origins of cells studied, and human donor characteristics should be specified. If complex preparations containing hiPSC-CMs are used (e.g., co-cultures, organoids, engineered heart tissues), descriptions of the protocols used in creating these preparations

			should be provided. For primary human cardiomyocyte preparations, the tissue sources, harvesting, isolation, and enrichment procedures followed should be described. Acceptable morphological and functional inclusion criteria for the preparations as well as electrophysiologic characteristics (including baseline action potential/field potential durations, spontaneous beat frequency and variability [if applicable], resting membrane potential, upstroke characteristics, conduction patterns and/or velocity) should be clearly defined. Estimates of the proportion of preparations fulfilling criteria should be included. • Technology platform: The methodologies used (e.g., transmembrane potential recordings [whole cell patch clamp, sharp electrode, or voltage-sensing dye approaches], extracellular recordings using field potentials, visual or impedance-based motion approaches, or calcium-sensing dyes) should be clearly described. The analysis package used for marking and interpreting waveforms should be described, with representative recordings (along with pertinent waveform markings) provided. A description of the plates or chambers used (including presence or absence of flow, substrate composition, recording electrode characteristics) should be provided.
2.4		What are important considerations when designing and implementing experimental protocols for *in vitro* myocyte repolarization studies?	Protocols should be designed to address a specific question (e.g., concentration-dependent effects on repolarization). The rationale of choosing single- or sequential- dose protocol should be provided. Bath temperature should be stable at physiologic (35–37 ºC) temperature. The sampling "window" for data collection should be clearly defined. Deviations from protocols should be clearly described, along with expected consequences. • For spontaneously beating preparations, changes in beating rate influence repolarization independent of direct drug effects on repolarizing currents. Spontaneous beat rates in the absence and presence of drugs should be clearly indicated along with the extent of drug-induced rate changes. The choice and justification of correction formula used when assessing repolarization effects in such preparations should be provided. Due to limitations of rate correction in spontaneously beating hiPSC-CMs,

			interpretation of potential repolarization changes may not be possible when a drug causes a rate change. • For paced preparations, the pacing protocol (pattern and duration) should be described as well as assurances given that the preparations followed external stimulation in the presence and absence of test compound. • To demonstrate recording quality, the study report should contain time-course plots of primary endpoints (demonstrating drug equilibration) and general stability of the preparations and signal recordings. • Concentration-dependent repolarization effects can be derived based on vehicle-corrected and/or baseline subtracted comparisons of drug vs. vehicle treated preparations. For higher throughput multi-well platforms, it is preferable to conduct vehicle and test drug studies on the same plate. The number of replicates (useful for evaluating reproducibility but not inferential statistical testing) should be reported. Power calculations are helpful to establish statistical sensitivity to repolarization endpoints. • It is important to characterize drug exposures during *in vitro* cardiomyocyte repolarization studies. For well-based studies, drug exposures could be verified using media sampled from test wells or from "satellite studies" (parallel studies using identical protocols and study conditions conducted without measuring electrophysiologic measurements). With continuous flow systems the sampling of effluent from test chambers is valuable for assessing drug exposures. Exposures should be presented as total drug concentration and free drug concentrations (if plasma protein binding characteristics for the media used is known).
2.5		How does one define biological sensitivity of a cardiomyocyte *in vitro* repolarization assay?	The electrophysiologic sensitivity of cardiomyocyte preparations should be calibrated with established positive controls to confirm their "fit for purpose" role in defining pharmacological block of cardiac ion channel(s). This is readily accomplished by constructing concentration-response curves with recognized and specific ion current blocking agents. • At minimum, it is important to characterize sensitivity to block of the prominent outward repolarizing current IKr/hERG with specific blocking agents (e.g., E-4031 or dofetilide) over relevant concentration ranges.

			• Block of the inward L-type calcium current (I_{CaL}) and late sodium current (I_{NaL}) may mitigate delayed repolarization. Demonstrating sensitivity to specific I_{CaL} (e.g., nifedipine or nisoldipine) and I_{NaL} (e.g., mexiletine or lidocaine) blocking agents is helpful for clarifying integrated cellular electrophysiological responses of multi-channel blocking drugs.

3. Best Practice Considerations for the *In vivo* QT Studies

#	Date of Approval	Questions	Answers
3.1		What are best practice considerations for species selection and general design of the (standard) *in vivo* QT study?	The most appropriate species should be selected and justified (ICH S7B, Section 3.1.3). It is preferable to use the same animal species in the safety pharmacology and non-rodent toxicity studies to facilitate understanding of the possible relationship between adverse cardiovascular pharmacodynamic effects and structural effects on the heart, and to obtain complementary information on systemic exposure level (toxicokinetics). While it is customary to use conscious freely-moving telemeterized animals for the *in vivo* QT studies, the choice of alternative model approaches (e.g., anesthetized or paced animals) might be justified in certain circumstances to achieve adequate exposures or to overcome specific compound-related challenges (e.g., changes in heart rate, tolerability or bioavailability limitations in conscious animals).
3.2		What should be considered for exposure assessment during the *in vivo* QT study?	The ICH S7B guideline states that drug exposures should include and exceed anticipated therapeutic concentrations. If the *in vivo* QT data are to be used as part of an integrated risk assessment for situations described in ICH E14 Q&As 5.1 & 6.1, the exposure should cover the anticipated high clinical exposure scenario (see S7B Q&A 1.1). An assessment of exposure in the same animals used for the pharmacodynamic assessment is encouraged. Sampling should take place at relevant timepoints and in a manner that limits interference with the pharmacodynamic effects. This could be done by sampling complete pharmacokinetic profiles in the same animals on a separate day after an adequate

			washout or by using limited samples from the pharmacodynamic assessment day to demonstrate consistency with full pharmacokinetic profiles generated in different animals in a separate study. In certain cases, the analysis of QTc interval together with adequate pharmacokinetic sampling makes it possible to perform dedicated exposure-response modeling similar to concentration-QT analysis for clinical QT studies. This can be helpful when the study should be powered to detect an effect similar to dedicated QT studies in humans (e.g., when using *in vivo* QT data as part of an integrated nonclinical and clinical risk assessment as described in ICH E14 Q&A 6.1). In addition, exposure-response modeling may be helpful in other circumstances when QT prolongation is observed or anticipated based on hERG assay results.
3.3		What information is needed to support the choice of heart rate correction method in an *in vivo* QT assay?	Optimally, the sponsor should demonstrate the independence of QTc to RR intervals observed in the study through QTc versus RR plots accompanied by additional information (e.g., number of matched QTc-RR pairs, correlation metric, 95% confidence intervals, p-values). QT-RR interval relationship is also important. Justification of correction factors used for QT measures should be provided when test drugs affect heart rate. In certain cases, individual QT correction based on QT-RR relationship is a preferred method as it is more accurate and sensitive than the general methods such as Bazett, Fridericia or Van de Water when the test drugs affect heart rate. The main reason for not using correction formulae based on historical data is the fixed rate correction coefficients. Non-rodent species show species-specific and individual differences in their QT-RR relationships.
3.4		How should the sensitivity of the assay be evaluated?	The test system used for an *in vivo* QT assay should provide a robust response. Assay sensitivity of relevant functional endpoints should be evaluated and reported to enable data interpretation (in supporting initiating first-in-human studies and/or an integrated nonclinical and clinical integrated risk assessment to be applied under scenarios in ICH E14 Q&As 5.1 or 6.1) and contextualization. Demonstration of assay sensitivity can be achieved by defining minimum detectable differences and testing the effects of positive controls. Statistical power calculations could also be

			provided from historical data from the same laboratory using the identical protocol. If historical positive control data are utilized to justify assay sensitivity or statistical power is calculated from historical control data, then the variance of the present data should be consistent with that seen historically. If study results are to be used to support an integrated nonclinical and clinical risk assessment described in ICH E14 Q&A 6.1, then the study should be powered to detect a QTc prolongation effect of a magnitude similar to dedicated clinical QT studies (see S7B Q&A 1.1).
3.5		What are the recommended conventions for presenting the pharmacodynamic and pharmacokinetic results of an *in vivo* QT assay?	To facilitate the regulatory review of an *in vivo* QT assay, the following are general recommendations that may vary case-by-case. Pharmacodynamic Content • Summary tables and figures showing absolute mean values, mean percent change from baseline, confidence intervals, and p-values for changes from baseline and vehicle control. Pharmacokinetic Content • Tabulations of summary statistics for Cmax, AUC, and Tmax for the parent drug and metabolites along with plasma concentration vs. time plots (if sufficient samples have been collected to support their calculation). Individual animal data should be provided.

4. Principles of Proarrhythmia Models

#	Date of Approval	Questions	Answers
4.1		The ICH S7B guideline (Section 3.1.4) states that directly assessing the proarrhythmic risk of pharmaceuticals that prolong the QT interval would be a logical	Different models, including *in silico, in vitro, ex vivo* and *in vivo* models, have the potential to be used as part of an integrated risk assessment strategy to evaluate the proarrhythmic risk of QT-prolonging pharmaceuticals in humans. Because these models have a common feature of using nonclinical experimental data as input and generating human proarrhythmia risk prediction as output, they can generally be

		undertaking and interested parties are encouraged to develop these models and test their usefulness in predicting risk in humans. What are general principles to evaluate whether a proarrhythmic risk prediction model could be used as part of an integrated risk assessment strategy?	referred to as proarrhythmia risk prediction models. The model input can vary among different models, for example, ion channel pharmacology data as input to *in silico* models, drug-induced changes in cellular repolarization and/or arrhythmia events as input to hiPSC-CM models, and drug-induced ECG changes as input to *ex vivo*/*in vivo* models. However, the model output (either discrete risk categories or continuous risk scores) is similar among different models. Such a feature makes it possible to develop generic principles for evaluating the predictivity of proarrhythmia risk prediction models without specifying the type of underlying experimental data as model input. The following general principles should be applied to all proarrhythmia risk prediction models intended to be used as part of an integrated risk assessment for regulatory purposes. While the main focus of these principles is to evaluate a model's predictivity of TdP risk, they are general enough to guide the development of models predicting different types of proarrhythmia. 1. A defined endpoint consistent with the context of use of the model. 2. A defined scope and limitations of the model. This includes the experimental protocols to generate model input (experimental data capturing pharmacological effect of drug), and the compounds tested should have the same arrhythmic mechanisms covered by the model. 3. A prespecified analysis plan and criteria to assess model predictivity. The analysis plan should include methods to separate the training and validation steps. In the training step, a series of reference compounds is used to adjust the model. In the validation step, another series of reference compounds is used to evaluate the performance of the pre-specified model. The reference compounds used for the training and validation steps should not overlap. 4. A fully disclosed algorithm to translate experimental measurements (model input) to proarrhythmia risk (model output), allowing independent reproduction of the model development process using the

			associated training and validation datasets to re-evaluate the model performance. 5. The uncertainty in the model inputs should be captured and propagated to the model predictions. The experimental variability associated with model input should be quantified using appropriate statistical methods and then translated into probabilities of the predicted risk. 6. A mechanistic interpretation of the model, which describes the relationship between the model inputs and mechanism for the arrhythmia.
4.2		Are there any additional considerations for the use of proarrhythmia risk prediction models?	After a proarrhythmia risk prediction model is developed, a process can be followed to evaluate whether or not the model development complied with the six principles in Q&A 4.1 above and to define the specific context of use of the model. Such a process is called model qualification (see Q&A 4.3 below about the qualification process). After a model is qualified, the use of such a model is not limited to the specific facility that submitted the qualification package. However, if another facility intends to use the qualified model, that facility should perform lab-specific validation and calibration of the model using a subset of the reference compounds that were originally used to develop the model.
4.3		How can a sponsor use a model for regulatory submission and what are the limitations?	Sponsors can use results from a qualified proarrhythmia model as one component in the totality of evidence approach to risk assessment under the context of use for which the model was developed and qualified. When a facility intends to use the model to produce data for regulatory submission, a set of control compounds should be tested to assess the consistency between the new data and the historical lab-specific validation data. Some regulators have procedures for the formal qualification of models, whereas others do not. Model developers are encouraged to contact a regulatory agency about the specific model qualification procedure. If a proarrhythmia model is included in a regulatory submission, proof of qualification of the model under the guidance of the general principles should be provided in an appendix to the study report. Supportive documentation could include published papers, if the included validation dataset is described in

			sufficient detail to allow an independent assessment. Importantly, the general principles for model qualification set forth in this Q&A will only support the use of a proarrhythmia risk prediction model as part of an integrated risk assessment that incorporates all relevant nonclinical and clinical information.