ICH S7B Guideline

Step 2 Revision

The Nonclinical Evaluation of the Potential for Delayed Ventricular Repolarization (QT Interval Prolongation) by Human Pharmaceuticals

June 10, 2004

1. INTRODUCTION 1
1.1 Objective of the Guideline 1
1.2 Background 1
1.3 Scope of the Guideline 2
1.4 General Principles 2
2. Guideline 2
2.1 Objectives of S7B Studies 2
2.2 Considerations for Selection and Design of Studies 2
2.3 Nonclinical Testing Strategy 3
2.3.1 *In vitro* I_{Kr} assay 3
2.3.2 *In vivo* QT assay 3
2.3.3 Chemical/pharmacological class 3
2.3.4 Relevant nonclinical and clinical information 4
2.3.5 Follow-up studies 4
2.3.6 Integrated risk assessment 4
2.3.7 Evidence of risk 5
2.4 Timing of S7B Nonclinical Studies and Integrated Risk Assessment in Relation to Clinical Development 5
3. Test Systems 5
3.1 Considerations for Test Systems 5
3.1.1 Use of positive control substances and reference compounds 5
3.1.2 *In vitro* electrophysiology studies 5
3.1.3 *In vivo* electrophysiology studies 7
3.1.4 Simulated pathological conditions and arrhythmias 8

The Nonclinical Evaluation of the Potential for Delayed Ventricular Repolarization (QT Interval Prolongation) by Human Pharmaceuticals

1. INTRODUCTION

The assessment of the effects of pharmaceuticals on ventricular repolarization and proarrhythmic risk is the subject of active investigation. When additional data (nonclinical and clinical) are accumulated in the future, they will be evaluated and this guideline might be revised.

1.1 Objective of the Guideline

This guideline describes a nonclinical testing strategy for assessing the potential of a test substance to delay ventricular repolarization. This guideline includes information concerning nonclinical assays and an integrated risk assessment.

1.2 Background

The QT interval (time from the beginning of the QRS complex to the end of the T wave) of the electrocardiogram (ECG) is a measure of the duration of ventricular depolarization and repolarization. QT interval prolongation can be congenital or acquired (e.g., pharmaceutical-induced). When ventricular repolarization is delayed and the QT interval is prolonged, there is an increased risk of ventricular tachyarrhythmia, including torsade de pointes, particularly when combined with other risk factors (e.g., hypokalemia, structural heart disease, bradycardia). Thus, much emphasis has been placed on the potential proarrhythmic effects of pharmaceuticals that are associated with QT interval prolongation.

Ventricular repolarization, determined by the duration of the cardiac action potential, is a complex physiological process. It is the net result of the activities of many membrane ion channels and transporters. Under physiological conditions, the functions of these ion channels and transporters are highly interdependent. The activity of each ion channel or transporter is affected by multiple factors including, but not limited to, intracellular and extracellular ion concentrations, membrane potential, cell-to-cell electrical coupling, heart rate, and autonomic nervous system activity. The metabolic state (e.g., acid-base balance) and location and type of cardiac cell are also important. The human ventricular action potential consists of five sequential phases:

- phase 0: The upstroke of the action potential is primarily a consequence of a rapid, transient influx of Na^+ (I_{Na}) through Na^+ channels.
- phase 1: The termination of the upstroke of the action potential and early repolarization phase result from the inactivation of Na^+ channels and the transient efflux of K^+ (I_{to}) through K^+ channels.
- phase 2: The plateau of the action potential is a reflection of a balance between the influx of Ca^{2+} (I_{Ca}) through L-type Ca^{2+} channels and outward repolarizing K^+ currents.
- phase 3: The sustained downward stroke of the action potential and the late repolarization phase result from the efflux of K^+ (I_{Kr} and I_{Ks}) through delayed rectifier K^+ channels.
- phase 4: The resting potential is maintained by the inward rectifier K^+ current (I_{K1}).

Prolongation of the action potential can result from decreased inactivation of the inward Na^+ or Ca^{2+} currents, increased activation of the Ca^{2+} current, or inhibition of one or more of the outward K^+ currents. The rapidly and slowly activating components of the delayed rectifier potassium current, I_{Kr} and I_{Ks}, seem to have the most influential role in determining the duration of the action potential and thus the QT interval. The human ether-a-go-go-related gene (hERG) and KvLQT1 gene encode pore-forming proteins that are thought to represent the α-subunits of the human potassium channels responsible for I_{Kr} and I_{Ks}, respectively. These α-subunit proteins can form hetero-oligomeric complexes with auxiliary β-subunits (i.e. MiRP and MinK gene products), which have been speculated to modulate the gating properties of the channel proteins. The most

common mechanism of QT interval prolongation by pharmaceuticals is inhibition of the delayed rectifier potassium channel that is responsible for I_{Kr}.

1.3 Scope of the Guideline

This guideline extends and complements the "ICH Guideline on Safety Pharmacology Studies for Human Pharmaceuticals" (ICH S7A). This guideline applies to new chemical entities for human use and marketed pharmaceuticals when appropriate (e.g., when adverse clinical events, a new patient population, or a new route of administration raises concerns not previously addressed). Pharmaceuticals for which testing is not called for are described in ICH S7A.

1.4 General Principles

Principles and recommendations described in ICH S7A also apply to the studies conducted in accordance with the present guideline.

In vitro and *in vivo* assays are complementary approaches; therefore, according to current understanding, both assay types should be conducted.

The investigational approach and evidence of risk should be individualized for the test substance, depending on its pharmacodynamic, pharmacokinetic and safety profiles.

2. GUIDELINE

2.1 Objectives of S7B Studies

The objectives of studies are to: 1) identify the potential of a test substance and its metabolites to delay ventricular repolarization, and 2) relate the extent of delayed ventricular repolarization to the concentrations of a test substance and its metabolites. The study results can be used to elucidate the mechanism of action and, when considered with other information, estimate risk for delayed ventricular repolarization and QT interval prolongation in humans.

2.2 Considerations for Selection and Design of Studies

Nonclinical methodologies can address the following:

- Ionic currents measured in isolated animal or human cardiac myocytes, cultured cardiac cell lines, or heterologous expression systems for cloned human ion channels,
- Action potential parameters in isolated cardiac preparations or specific electrophysiology parameters indicative of action potential duration in anesthetized animals,
- ECG parameters measured in conscious or anesthetized animals,
- Proarrhythmic effects measured in isolated cardiac preparations or animals.

As indicated above, these four functional levels can be investigated by *in vitro* and/or *in vivo* methods. Findings from the first three functional levels listed above are considered useful and complementary. The value of proarrhythmia models is discussed in section 3.1.4.

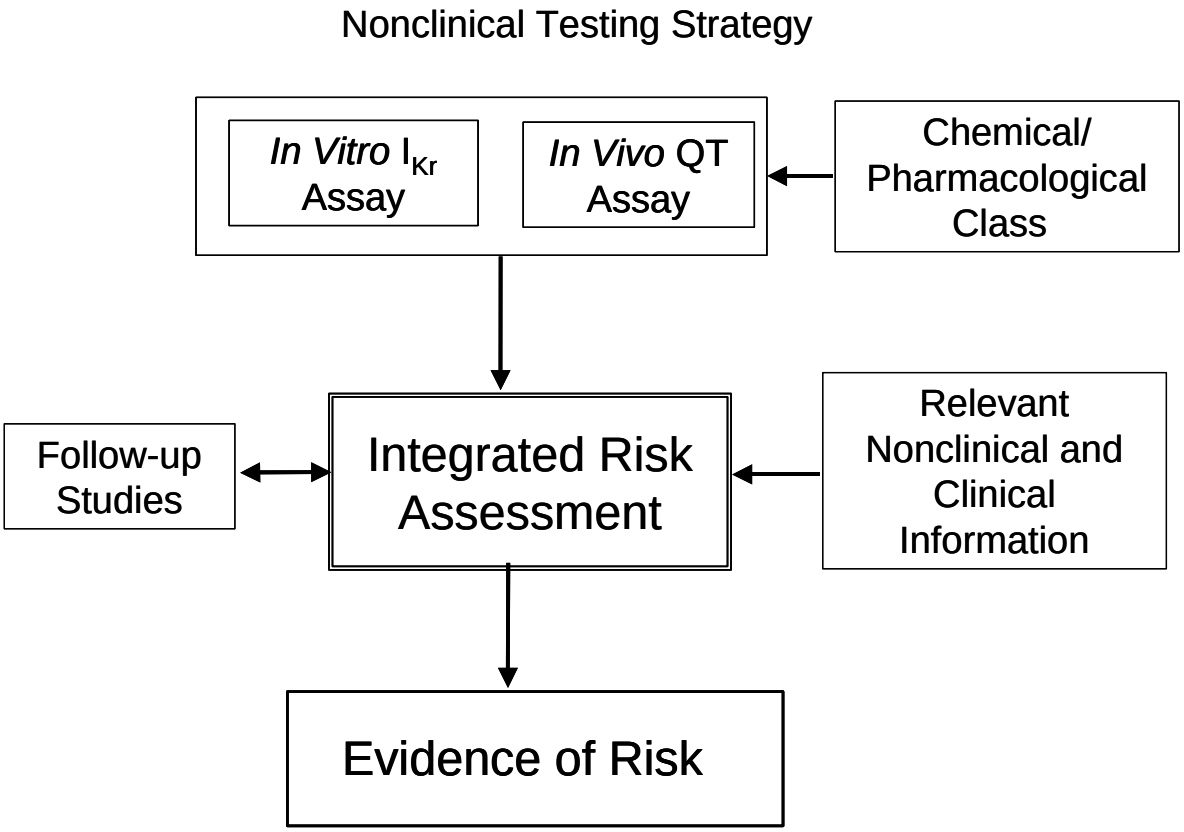
In vitro electrophysiology studies can explore potential cellular mechanisms that might not be evident from *in vivo* data. Changes in other cardiovascular parameters or effects on multiple ion channels can complicate interpretation of data. Complementary assessments in other systems can address this issue. Although delay of repolarization can occur through modulation of several types of ion channels, inhibition of I_{Kr} is the most common mechanism responsible for pharmaceutical-induced prolongation of QT interval in humans.

Experimental models that possess the full complement of mechanisms can be more informative with regard to the clinical situation. Carefully designed and conducted *in vivo* studies allow evaluation of metabolites and can enable estimation of safety margins. *In vivo* ECG evaluations provide information on conduction properties and non-cardiac influences (e.g., autonomic

nervous system tone). Studies of action potential parameters provide information on the integrated activity of multiple ion channels in the heart.

2.3 Nonclinical Testing Strategy

The following sections describe a general nonclinical testing strategy for assessing risk for delayed ventricular repolarization and QT interval prolongation that is pragmatic and based on currently available information. The figure illustrates the component elements of the testing strategy, but not specific test systems or their designs.



2.3.1 *In vitro* I_{Kr} assay

Results from an assay that evaluates effects on I_{Kr} or the ionic current through a native or expressed I_{Kr} channel protein, such as that encoded by hERG (see section 3.1.2).

2.3.2 *In vivo* QT assay

Results from an *in vivo* assay that measures indices of ventricular repolarization such as QT interval (see section 3.1.3).

2.3.3 Chemical/pharmacological class

Consideration should be given to whether the test substance belongs to a chemical/pharmacological class in which some members have been shown to induce QT interval prolongation in humans (e.g., antipsychotics, histamine H-1 receptor antagonists, fluoroquinolones). This should, where appropriate, influence the choice of reference compound(s) and be included in the integrated risk assessment.

2.3.4 Relevant nonclinical and clinical information

Additional information for the integrated risk assessment can include results from:

- Pharmacodynamic studies,
- Toxicology/safety studies,
- Pharmacokinetic studies, including plasma levels of parent substance and metabolites (including human data if available),
- Drug interaction studies,
- Tissue distribution and accumulation studies,
- Post-marketing surveillance.

2.3.5 Follow-up studies

Follow-up studies are intended to provide greater depth of understanding or additional knowledge regarding the potential of test substance for delayed ventricular repolarization and QT interval prolongation in humans. Such studies can provide additional information concerning potency, mechanism of action, slope of the dose-response curve, or magnitude of the response. Follow-up studies are designed to address specific issues, and, as a result, various *in vivo* or *in vitro* study designs can be applicable.

In circumstances where results among nonclinical studies are inconsistent and/or results of clinical studies differ from those for nonclinical studies, retrospective evaluation and follow-up nonclinical studies can be used to understand the basis for the discrepancies. Results from follow-up studies can be a significant component of an integrated risk assessment.

Relevant nonclinical and clinical information along with the following should be considered in the selection and design of follow-up studies:

- Use of ventricular repolarization assays that measure action potential parameters in isolated cardiac preparations (see section 3.1.2),
- Use of specific electrophysiological parameters indicative of action potential duration in anesthetized animals (see section 3.1.3),
- Repeated administration of test substance,
- Selection of animal species and gender(s),
- Use of metabolic inducers or inhibitors,
- Use of concurrent positive control substances and reference compounds (see section 3.1.1),
- Inhibition of other channels not previously evaluated,
- Measurement of electrophysiological parameters at multiple time points,
- Confounding effects in conscious animals that limit the interpretation of data such as test substance-induced effects on heart rate or autonomic tone, or toxicities such as tremor, convulsion, or emesis.

2.3.6 Integrated risk assessment

The integrated risk assessment is the evaluation of non-clinical study results including the results from follow-up studies and other relevant information. The integrated risk assessment should be scientifically based and individualized for the test substance. Such an assessment can contribute to the design of clinical investigations and interpretation of their results. The integrated risk assessment should be provided for the Investigator's Brochure and the Nonclinical Overview (ICH M4). The integrated risk assessment should also consider:

- Potencies of test substance in S7B assays relative to reference compound(s),
- Safety margins from *in vivo* QT assays,
- Assay sensitivity and specificity,
- Contribution of metabolites to QT interval prolongation as well as metabolic differences between humans and animals.

2.3.7 Evidence of risk

Evidence of risk is the overall conclusion from the integrated risk assessment for a test substance to delay ventricular repolarization and prolong QT interval in humans.

2.4 Timing of S7B Nonclinical Studies and Integrated Risk Assessment in Relation to Clinical Development

Results from S7B nonclinical studies assessing the risk for delayed ventricular repolarization and QT interval prolongation generally do not need to be available prior to first administration in humans. However, these results, as part of an integrated risk assessment, can support the planning and interpretation of subsequent clinical studies. The early availability of these data is considered valuable.

3. TEST SYSTEMS

3.1 Considerations for Test Systems

This section provides an overview of methodologies currently used to assess the potential for a test substance to delay ventricular repolarization and to prolong QT interval. The following criteria should be considered in selecting the most appropriate test systems:

- Assay methodology and experimental endpoints are scientifically valid and robust,
- Assays and preparations are standardized,
- Results are reproducible,
- Endpoints/parameters of the assays are relevant for assessing human risk.

3.1.1 Use of positive control substances and reference compounds

Positive control substances should be used to establish the sensitivity of *in vitro* preparations for ion channel and action potential duration assays. In the case of *in vivo* studies, positive control substances should be used to validate and define the sensitivity of the test system, but need not be included in every experiment.

For test substances belonging to a chemical/pharmacological class that is associated with QT interval prolongation in humans, the use of concurrent reference compound(s) (member(s) of the same class) in *in vitro* and *in vivo* studies should be considered to facilitate ranking the potency of the test substance in relation to its comparators.

Whether or not positive control substances or reference compounds are used in experiments should be justified.

3.1.2 *In vitro* electrophysiology studies

In vitro electrophysiology studies can provide valuable information concerning the effect of a test substance on action potential duration and/or cardiac ionic currents. These assays have an important role in assessing the potential for QT interval prolongation and elucidating cellular mechanisms affecting repolarization. *In vitro* electrophysiology studies employ either single cell (e.g., heterologous expression systems, disaggregated cardiomyocytes) or multicellular (e.g.,

Purkinje fiber; papillary muscle; trabeculae; perfused myocardium; intact heart) preparations. Multicellular preparations are stable test systems to study action potential duration. While more fragile, single cell preparations minimize diffusional barriers to the site of action. The analysis of parameters for each phase of the action potential such as V_{max} for phase 0 (I_{Na}), APD_{30} for phase 2 (I_{Ca}) and "triangulation" for phase 3 (I_K) can be useful to investigate the effects on specific channels responsible for these phases. In addition, some parameters derived from the Langendorff preparation have been reported to provide information regarding proarrhythmia. Heterologous expression systems, where human ion channel protein(s) are expressed in noncardiac cell lines, are used to assess the effects of a test substance on a specific ion channel. Disaggregated myocytes are technically more challenging than the expression systems but have the advantage of being suitable for assessing effects on both action potential duration and ionic currents.

Tissue and cell preparations for *in vitro* assays are obtained from different laboratory animal species including rabbit, ferret, guinea pig, dog, swine, and occasionally from humans. The ionic mechanisms of repolarization in adult rats and mice differ from larger species, including humans (the primary ion currents controlling repolarization in adult rats and mice is I_{to}); therefore, use of tissues from these species is not considered appropriate. Species differences in terms of which cardiac ion channels contribute to cardiac repolarization and to the duration of the action potential should be considered in selecting a test system. When native cardiac tissues or cells are used, the characteristics and source of the preparation should be considered because the distribution of ion channel types varies according to the region and type of cell.

Test substance concentrations for *in vitro* studies should span a broad range, covering and exceeding the anticipated maximal therapeutic plasma concentration. Ascending concentrations should be tested until a concentration-response curve has been characterized or physicochemical effects become concentration-limiting. Ideally, the duration of exposure should be sufficient to obtain steady-state electrophysiological effects, unless precluded by the viability of the cell or tissue preparation. The duration of exposure should be indicated. Appropriate positive control substances should be used to establish the sensitivity of the *in vitro* assay system as well as to confirm that the ion channels of interest are present and stable.

Factors that can confound or limit the interpretation of *in vitro* electrophysiology studies include the following:

- The testing of high concentrations of the test substance can be precluded by limited solubility in aqueous physiological salt solutions,
- Adsorption to glass or plastic or non-specific binding to the test matrix can reduce the concentration of the test substance in the incubation or perfusion medium,
- Test substance concentrations can be limited by cytotoxic or physicochemical attributes of the test substance that disrupt cell membrane integrity so that electrophysiological endpoints cannot be obtained,
- Cardiac cells and tissues have limited capacity for drug metabolism and therefore *in vitro* studies using the parent substance do not provide information on the effects of metabolites. When *in vivo* nonclinical or clinical studies reveal QT interval prolongation that is not corroborated by *in vitro* studies using the parent substance, testing metabolites in the *in vitro* test systems should be considered.

High throughput potassium channel assays are being developed. While novel ion channel activity assays can be useful in preliminary screening of test substances to identify lead candidates for further electrophysiological testing, more experience will establish whether they have sufficient predictive value to be an alternative to voltage clamp assays.

Another screening approach is the use of competition binding protocols in which test substances are studied for their ability to displace a radiolabeled hERG channel blocker from a cell line expressing hERG. However, competition for radioligand-binding sites provides no information on agonistic or antagonistic effects of the test substance on I_{Kr}. Moreover, this assay will not identify

test substances that bind to hERG at sites other than the radioligand binding sites. Based upon these potential limitations, this assay is not considered a substitute for voltage clamp assays described above.

3.1.3 *In vivo* electrophysiology studies

Intact animal models allow investigation of ventricular repolarization or associated arrhythmias where integrated effects on the full complement of ion channel and cell types are assessed. Also, potential neuronal and hormonal influences on the pharmacodynamic effect of the pharmaceuticals are present in animals.

The QT interval of the ECG is the most commonly used endpoint to gauge effects of a test substance on ventricular repolarization. In specialized electrophysiology studies, regional information regarding the ventricular repolarization (e.g., monophasic action potential duration and effective refractory period) can also be obtained from *in vivo* models. Additional safety parameters of interest, including blood pressure, heart rate, PR interval, QRS duration, the presence of U waves, and arrhythmias, can be assessed simultaneously.

The QT interval and heart rate have an inverse, non-linear relationship, which varies among species, between animals, or even within the same animal at different heart rates. Thus, a change in heart rate exerts an effect on QT interval, which can confound the assessment of the effect of the test substance on ventricular repolarization and the QT interval. There are two important situations where there is variability in heart rate among animals: one is due to difference in autonomic tone, and the other is due to effects of test substances on heart rate. Therefore, the interpretation of data from *in vivo* test systems should take into account the effect of coincident changes in heart rate. Ideally, QT interval data obtained after administration of a test substance should be compared with control and baseline data at similar heart rates. When the variability is not due to the test substance, it can be reduced by training, or the use of anesthetized animal models. When the effects are due to test substances, the most common approach is to correct the QT interval for heart rate (QTc) using formulae such as Bazett or Fridericia; however, these corrections can yield misleading data, especially when differences in heart rate between treatment and control are large. An alternative approach is to maintain a constant heart rate using cardiac pacing.

Laboratory animal species used for *in vivo* electrophysiology studies include dog, monkey, swine, rabbit, ferret, and guinea pig. The ionic mechanisms of repolarization in adult rats and mice differ from larger species, including humans (the primary ion currents controlling repolarization in adult rats and mice is I_{to}); therefore, use of these species is not considered appropriate. The most appropriate *in vivo* test systems and species should be selected and justified.

The dose range should be in accord with that discussed in ICH S7A and, whenever feasible, should include and exceed the anticipated human exposure. The dose range can be limited by animal intolerance to the test substance, e.g., emesis, tremor, or hyperactivity. For studies designed to relate the extent of delayed ventricular repolarization to concentrations of the parent test substance and its metabolites, controlled exposure via constant intravenous infusion can be used. Monitoring exposure to the test substance and metabolites (see ICH S3A) provides opportunities to interpret dose- and concentration-response data and to design follow-up studies, if appropriate.

Factors that should be considered in conducting studies and interpreting the results include the following:

- Data acquisition and analysis methods,
- Sensitivity and reproducibility of the test systems,
- Dosing period and measurement points,
- Heart rate and other cardiovascular effects that confound interpretation of QT interval data,

- Inter-species and gender differences, e.g., cardiac electrophysiology, hemodynamics, or metabolism of pharmaceuticals,

- Pharmaceuticals that have effects on several ion channels can yield complex dose-response relationships that could be difficult to interpret.

3.1.4 Simulated pathological conditions and arrhythmias

The precise relationship between test substance-induced delay of ventricular repolarization and risk of proarrhythmia is not known. Directly assessing the proarrhythmic risk of pharmaceuticals that prolong the QT interval would be a logical undertaking; however, modeling of the clinical condition where pharmaceuticals elicit arrhythmia is complicated. Indices of proarrhythmic activity (e.g. electrical instability and temporal and/or spatial dispersion of refractoriness, reverse use-dependency, changes in action potential configuration) and animal models might have utility in assessing proarrhythmia. Interested parties are encouraged to develop these models and test their usefulness in predicting risk in humans.