

ICH S7B Guideline

Step 2 Revision

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

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1 The Nonclinical Evaluation of the Potential for Delayed Ventricular 2 Repolarization (QT Interval Prolongation) by Human Pharmaceuticals

3 1. INTRODUCTION

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

7 1.1 Objective of the Guideline

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

11 1.2 Background

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

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

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

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

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

49 **1.3 Scope of the Guideline**

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

55 **1.4 General Principles**

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

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

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

62 **2. GUIDELINE**

63 **2.1 Objectives of S7B Studies**

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

69 **2.2 Considerations for Selection and Design of Studies**

70 Nonclinical methodologies can address the following:

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

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

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

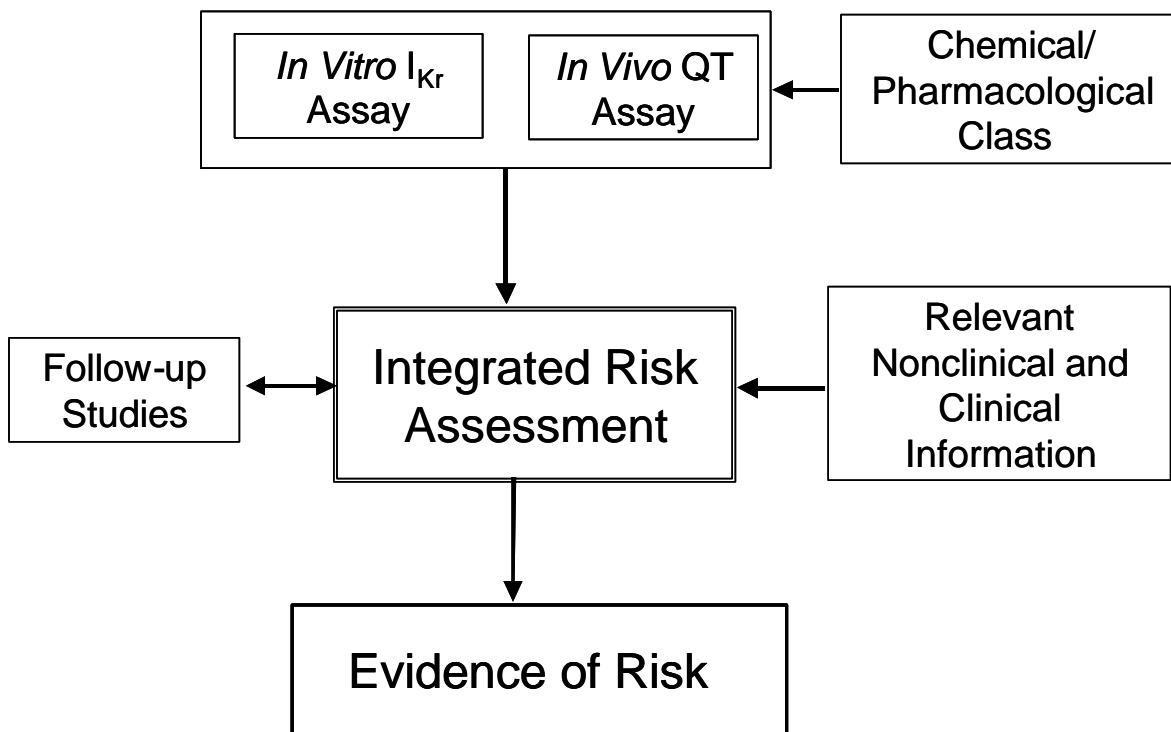
86 Experimental models that possess the full complement of mechanisms can be more informative
87 with regard to the clinical situation. Carefully designed and conducted *in vivo* studies allow
88 evaluation of metabolites and can enable estimation of safety margins. *In vivo* ECG evaluations
89 provide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

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

92 **2.3 Nonclinical Testing Strategy**

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

Nonclinical Testing Strategy



97

98 **2.3.1 *In vitro* I_{Kr} assay**

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

101 **2.3.2 *In vivo* QT assay**

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

104 **2.3.3 Chemical/pharmacological class**

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

110 **2.3.4 Relevant nonclinical and clinical information**

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

- 112 • Pharmacodynamic studies,
- 113 • Toxicology/safety studies,
- 114 • Pharmacokinetic studies, including plasma levels of parent substance and metabolites
115 (including human data if available),
- 116 • Drug interaction studies,
- 117 • Tissue distribution and accumulation studies,
- 118 • Post-marketing surveillance.

119 **2.3.5 Follow-up studies**

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

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

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

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

145 **2.3.6 Integrated risk assessment**

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

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

157 **2.3.7 Evidence of risk**

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

160 **2.4 Timing of S7B Nonclinical Studies and Integrated Risk Assessment in Relation to** 161 **Clinical Development**

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

167 **3. TEST SYSTEMS**

168 **3.1 Considerations for Test Systems**

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

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

176 **3.1.1 Use of positive control substances and reference compounds**

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

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

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

187 **3.1.2 *In vitro* electrophysiology studies**

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

193 Purkinje fiber; papillary muscle; trabeculae; perfused myocardium; intact heart) preparations.
194 Multicellular preparations are stable test systems to study action potential duration. While more
195 fragile, single cell preparations minimize diffusional barriers to the site of action. The analysis of
196 parameters for each phase of the action potential such as V_{max} for phase 0 (I_{Na}), APD_{30} for phase
197 2 (I_{Ca}) and “triangulation” for phase 3 (I_K) can be useful to investigate the effects on specific
198 channels responsible for these phases. In addition, some parameters derived from the
199 Langendorff preparation have been reported to provide information regarding proarrhythmia.
200 Heterologous expression systems, where human ion channel protein(s) are expressed in
201 noncardiac cell lines, are used to assess the effects of a test substance on a specific ion channel.
202 Disaggregated myocytes are technically more challenging than the expression systems but have
203 the advantage of being suitable for assessing effects on both action potential duration and ionic
204 currents.

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

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

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

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

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

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

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

247 **3.1.3 *In vivo* electrophysiology studies**

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

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

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

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

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

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

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

- 292 • Inter-species and gender differences, e.g., cardiac electrophysiology, hemodynamics, or
293 metabolism of pharmaceuticals,
- 294 • Pharmaceuticals that have effects on several ion channels can yield complex dose-response
295 relationships that could be difficult to interpret.

296 **3.1.4 Simulated pathological conditions and arrhythmias**

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

305