# Chapter 11
# Safety Pharmacology: Guidelines S7A and S7B *

**John E. Koerner and Peter K.S. Siegl**

**Abstract** Safety pharmacology studies are performed during nonclinical drug development to identify and characterize, in relationship to exposure, potentially undesirable pharmacodynamic effects of a substance on physiological functions. A major objective of these studies is to assess the relevance of these pharmacodynamic activities for human safety. The International Conference on Harmonisation (ICH) issued guidelines describing nonclinical safety pharmacology testing strategies to detect effects on core systems, that is, cardiovascular, respiratory, and central nervous systems (ICH S7A), and risk of delaying ventricular repolarization (QT interval prolongation) (ICH S7B). An ICH Expert Working Group (EWG) took on the task of developing safety pharmacology guidelines and achieved step 4 with ICH S7A in 2001. Drug-induced delay in ventricular repolarization (QT interval prolongation) is the topic of a complementary guideline, ICH S7B, which had many of the same EWG members and achieved step 4 in 2005. The present chapter describes these guidelines along with background and context for the final recommendations in the guidelines.

* This article reflects the personal opinions of the author and does not necessarily reflect those of the FDA.

J.E. Koerner, Ph.D. (✉)
United States Food and Drug Administration, Center for Drug Evaluation and Research, Silver Spring, MD, USA
e-mail: John.Koerner@fda.hhs.gov

P.K.S. Siegl
Siegl Pharma Consulting LLC, Blue Bell, PA, USA

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## 11.1 Introduction

Safety pharmacology originated as a scientific discipline, based on the observation that, in addition to findings in toxicology studies, pharmacodynamic (functional) effects can have clinical safety significance (Bass et al. 2004). These effects may not be readily captured in traditional toxicology studies. It was noted that "The adverse drug reactions [that] the standard toxicological test procedures do not aspire to recognize include most of the functional side effects. Clinical experience indicates, however, that these are much more frequent than the toxic reaction due to morphological and biochemical lesions…" (Zbinden 1979). Additionally, the origin of safety pharmacology guidelines was recently described by Pugsley et al. (2008). Regulatory authorities and sponsors had a common interest in being able to capture pharmacodynamic effects in nonclinical studies that are not captured in traditional toxicology studies.

The ICH safety pharmacology guidelines, S7A "Safety Pharmacology Studies for Human Pharmaceuticals" and S7B "Nonclinical Evaluation of the Potential for Delayed Ventricular Repolarization (QT Interval Prolongation) by Human Pharmaceuticals," describe nonclinical testing strategies to detect and characterize pharmacological activities of potential drug candidates that could impact clinical safety.[1] Since biological systems are complex, the ICH S7A and S7B guidelines emphasize testing for pharmacodynamic (functional) activities of drug candidates using in vivo testing models in which indices of vital organ function are evaluated. Although drug candidates are typically optimized for their therapeutic potential via high potency and selectivity at the therapeutic target, the drug candidate may have additional functional pharmacological activities not revealed in the lead optimization process. The types of pharmacological activities detected in safety pharmacology assays are also not typically evaluated in routine toxicology studies but do have direct corollaries to safety endpoints monitored in clinical studies.

The first reference to safety pharmacology studies in ICH guidelines was in ICH M3, "Guidance on Nonclinical Safety Studies for the Conduct of Human Clinical Trials and Marketing Authorization for Pharmaceuticals." An ICH Expert Working Group (EWG) took on the task of developing a safety pharmacology guideline and achieved step 4 with ICH S7A in 2001. The topic of assessing drug-induced delay in ventricular repolarization (QT interval prolongation) is the topic of a complimentary guideline, ICH S7B, which had many of the same EWG members and reached step 4 in 2005. It has been 6–10 years since the safety pharmacology guidelines were adopted by all three regions.

Developing these two guidelines was controversial because, with the exception of the Japanese Guidelines for General Pharmacology Studies (Ministry of Health, Labour and Welfare of Japan [MHLW]) (Anon 1995), safety pharmacology (also referred to as general pharmacology or ancillary pharmacology) was a function already performed by many sponsors to reduce risk of attrition in drug development.

[1] FDA refers to these guidelines as guidances in accordance with FDA's good guidance practice (62 FR 8961, February 27, 1997).

The timing, design, and types of studies varied among sponsors, reflecting different philosophies and risk tolerance (Bass et al. 2004). In some cases, sponsors considered their approach to be a competitive advantage. The goal of the ICH EWG was to create guidelines that provided direction for sponsors but also maintained flexibility.

## 11.2 Objectives and General Principles of Safety Pharmacology in Drug Discovery and Development Programs

A primary goal of safety pharmacology studies is to protect clinical trial participants and patients. Information from these studies can also aid in selection of the clinical candidates, doses, and design of clinical programs as well as reduce risk of attrition due to drug-related adverse effects during all phases of development. To accomplish this most effectively and to minimize use of resources and animals, the safety pharmacology guidelines recommend a scientific and efficient approach in the choice and design of assays, as well as interpretation of results (see Table 11.1). Safety pharmacology studies are usually performed during characterization of a development candidate and prior to initiation of clinical studies. At this stage there are data on selectivity from in vitro screens (receptors, enzymes, and ion channels), metabolism, and characterization of the targeted pharmacological activity.

### *11.2.1 Safety Pharmacology Assays*

Sponsors are encouraged to consider assays to evaluate other organ systems and/or endpoints based upon knowledge of selectivity profile and chemical/pharmacological class of the development candidate. In each guideline there is a recommended core battery of assays. These core assays (cardiovascular, central nervous, and respiratory systems) were considered to be vital, since adverse effects on their functions can be acutely life threatening. Results from core assays provide a standard set of data and are expected to be included in regulatory documents, unless there is justification for not doing these assays. In addition to data from the core battery, the sponsor should consider whether further characterization of the activity in other organ systems or with follow-up studies will provide more complete information for a better risk assessment. In this way, the EWG encouraged sponsors to gather information that can most effectively characterize the safety of the drug candidate.

Both guidelines encourage use of conscious, unrestrained animals based upon the premise that autonomic reflexes are intact and this setting is therefore "more physiological." Also use of conscious animals is analogous to assessing responses in conscious human subjects. The EWG understood that there could be direct pharmacological activity that is more easily detected in anesthetized preparations or

**Table 11.1** Steps in scientific approach to profiling drug candidates with safety pharmacology studies

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| 1. Identify mechanism and non-mechanism-based pharmacological activities of drug candidates in major organ systems using functional endpoints (in vivo). |
| 2. Characterize these pharmacological activities:<br>a. Relative potency—dose and concentration relative to human.<br>b. Mechanism of action. |
| 3. Compare activity and potency to reference drugs with clinical experience. |
| 4. Estimate relative risk of these activities for potential adverse effects in humans using all available data (safety pharmacology, toxicology, metabolism and clinical experience, target patient population, and concomitant medications). |

in vitro models and, consequently, one should not dismiss results from in vitro or anesthetized preparations when they do not appear to be consistent with results in conscious preparations. The guidance allowed for safety pharmacology endpoints to be captured in toxicology studies, which also utilize conscious animals—with the caveat that these core parameters need to be captured in a sensitive manner.

As in general toxicology studies, normal, healthy animals are used in safety pharmacology studies, because this provides the most consistent background for detecting, characterizing, and comparing pharmacological activities. In some cases, animal models of disease may be utilized as follow-up assays to aid in the overall risk assessment. This option was not specifically discussed in the guidelines due to concerns of applicability of animal disease models to broad patient populations. In part, the appropriateness of the specific disease model needs to be justified—and its ability (sensitivity) to capture clinically relevant effects needs to be demonstrated. Use of animal models of disease should be accompanied by data in healthy animals.

An option to conduct safety pharmacology assessments as part of a toxicology study is offered in both ICH S7A and ICH S7B, with the recommendation that assay sensitivity, validation, and quality of data be satisfactory in the toxicology study. The application of this option to assess risk of delayed ventricular repolarization (QT interval prolongation) is discussed below (Section “In Vivo QT Assay”).

### *11.2.2 Timing of Safety Pharmacology Studies*

The safety pharmacology guidelines recommend that the sponsor evaluate test compounds in the core batteries prior to initiation of clinical studies to provide support for first-in-human trials. Sponsors can perform additional nonclinical safety pharmacology studies later during development, for example, to help characterize unanticipated activity observed in toxicology or clinical studies. The guideline encourages sponsors to integrate safety pharmacology findings with those from toxicology, pharmacokinetic, and nonclinical and clinical pharmacology studies for interpretation of overall safety and risk assessments. It is also

important to reexamine safety pharmacology data as well as to consider performing additional studies during development as nonclinical and clinical data accumulate.

### *11.2.3 Frequency of Dosing, Route of Administration, and Dose Levels*

The guidelines recommend acute studies, with a single administration of the test substance via the intended clinical route in healthy animals. The guidelines also recommend that the sponsor determine the time course and dose–response relationship of drug-related effects. These recommendations are consistent with the primary objective of the studies, which is to determine pharmacodynamic (functional) effects of the test substance. The safety pharmacology studies are not intended to mimic the clinical situation or to evaluate how the response is modified by disease. Like toxicology studies, these experimental conditions were chosen as the most consistent setting to detect and characterize pharmacological activities. Implications/consequences of the safety pharmacology actions in humans with or without disease should be considered in the risk assessment (see Table 11.1).

The recommendation for dose levels in safety pharmacology studies parallels the approach in toxicology studies. That is, drugs will have toxicity or unanticipated pharmacological activity at some dose level. The goal of these studies is to identify and characterize dose-limiting pharmacodynamic effects and to determine safety margins to guide clinical testing. Although not required, information on the mechanism(s) of the unanticipated safety pharmacology activity can help in the overall risk assessment by enabling comparisons with drugs sharing the same mechanism(s). Because safety margins can change as additional information becomes available, that is, when therapeutic doses are refined with clinical data, margins should be reevaluated when appropriate.

### *11.2.4 Assay Sensitivity and Use of Reference Compounds*

An important scientific aspect of recommendations in the safety pharmacology guidelines is to interpret and communicate the results in the context of the assay used. Both guidelines encourage reporting data in reference to assay sensitivity and responses to positive and negative controls (see box below).

Reporting that a test compound has no activity in an assay without knowledge of the assay's sensitivity and whether positive controls can be detected is a poor use of resources, and the conclusion can be misleading. It is incorrect to assume that assays employing similar protocols will perform exactly the same in every laboratory, even if performed under Good Laboratory Practices (GLP). To be able to conclude that there is no activity with the test compound in an assay that has a, say, 90 % power to

**Text from Safety Pharmacology Guidelines on Assay Sensitivity and Use of Positive and Negative Controls**

ICH S7A

*"Appropriate negative and positive control groups should be included in the experimental design. In well-characterized in vivo test systems, positive controls may not be necessary. The exclusion of controls from studies should be justified."*

ICH S7B

*"A sub-maximally effective concentration of a positive control substance should be used to demonstrate the responsiveness of in vitro preparations for ion channel and action potential duration assays and should be included in every study. 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 study."*

detect a change of 10 % magnitude and where a clinically relevant positive control can be detected in the assay at a relevant exposure is much more useful than merely concluding that the test compound had no activity in the assay. The EWG recognized that compounds have additional activities at some level; therefore, the goal is identify activity and to report the conditions at which no activity was observed. A common error is to evaluate high doses or concentrations of the positive control (e.g., high dose of dofetilide for QT interval prolongation or $I_{Kr}$/hERG inhibition); assessment of excessive doses does not adequately assess assay sensitivity. There was much discussion about the need for positive controls, and a compromise was reached based on scientific need, practicality, and animal usage. In general, concurrent positive controls were recommended for in vitro studies, whereas for in vivo studies, it is reasonable to rely on historical control data for that laboratory.

### *11.2.5 Relationship Between Pharmacodynamic and Pharmacokinetic Data*

To satisfy the recommendation in the guidelines that exposure of drug and metabolites will include and exceed targeted exposure in humans, plasma levels of drug and metabolites need to be documented for the dose levels tested. While it is ideal to measure pharmacodynamic (PK) and pharmacokinetic (PD) in same animals to minimize variability, this may not be practical; therefore, PK data from other studies are sometimes used. Note that the use of extrapolated PK values to document exposure can be misleading and result in erroneous estimates of safety margins.

Using such data to support results from safety pharmacology studies is inconsistent with the recommendations in the guidelines.

Because pharmacokinetics and metabolism can differ among species, it is prudent to examine the magnitude and timing of PD effects in relation to plasma levels of parent and, if appropriate, metabolites. This is consistent with interrogating direct pharmacological activities and determination of safety margins. When there is a direct correspondence between time courses of activity and plasma levels, as well as a concentration/dose and magnitude of effect, it strengthens the conclusion that the observed effect is test article related. Reporting activity in terms of plasma levels also facilitates translation of relative potencies (e.g., $ED_{50}$ or $IC_{50}$ concentrations) and thresholds for activity (e.g., NOEL or NOAEL) among species, including humans.

### *11.2.6 Safety Pharmacology Studies with Biologics*

In ICH S7A, "For biotechnology-derived products that achieve highly specific receptor targeting, it is often sufficient to evaluate safety pharmacology endpoints as a part of toxicology and/or pharmacodynamic studies; therefore, safety pharmacology studies can be reduced or eliminated for these products." This is consistent with guidance provided in ICH S6 (Preclinical Safety Evaluation of Biotechnology-Derived Pharmaceuticals): "It is important to investigate the potential for undesirable pharmacological activity in appropriate animal models and, where necessary, to incorporate particular monitoring for these activities in the toxicity studies and/or clinical studies. … These functional indices may be investigated in separate studies or incorporated in the design of toxicity studies."

### *11.2.7 Good Laboratory Practice*

Both guidelines point out the importance of ensuring the reliability and quality of the nonclinical safety pharmacology studies because the data are used to support clinical safety. It is noted that "this is normally accomplished through conduct of studies in compliance with good laboratory practice (GLP)." There are situations, however, where the one or more of the core battery safety pharmacology studies might have been performed at a development stage before it was practical to satisfy all aspects of GLP. The guideline indicated that "data quality and integrity in safety pharmacology studies should be ensured even in the absence of formal adherence to the principles of GLP. When studies are not conducted in compliance with GLP, study reconstruction should be ensured through adequate documentation of study conduct and archiving of data."

## 11.3 S7A Guideline "Safety Pharmacology Studies for Human Pharmaceuticals"

In 1991, Japan's Ministry of Health and Welfare (MHLW) issued a Guideline for General Pharmacology. This guideline recommended evaluation of drug candidates in a panel of in vitro and in vivo assays to assess direct pharmacological activity of drug candidates on many vital functions including the autonomic nervous system (Anon 1995). There were no similar guidelines issued by regulatory agents in other regions. Most sponsors appreciated the value of general or safety pharmacology studies to support selectivity of drug candidates and design of early clinical trials; however, the strategy varied among sponsors based upon their experience and risk tolerance (Bass et al. 2004). The goal of the ICH S7 EWG was to develop a guideline that provided practical direction consistent with the objectives in ICH M3 for studies recommended to be performed prior to initiating clinical studies. For sponsors already doing these type studies, the core battery was usually a portion of their packages, and, for those sponsors who were not doing these studies, the guideline was to facilitate their accomplishing this goal. A key recommendation from the EWG was that the core safety pharmacology studies should be performed before initiating clinical studies to aid in both design of and interpretation of results from the clinical development program. A key goal was to provide for an additional measure of safety in the first-in-human study.

The EWG did not include the in vitro studies from the MHLW guideline in S7A guidance because they wanted to provide flexibility in how sponsors handled in vitro selectivity screening. The ICH S7A guideline refers to in vitro data and recommends using results from the in vitro studies to select and design the safety pharmacology studies. By choosing to focus on evaluation of functional endpoints for vital organ systems, the results from the safety pharmacology studies should reflect the consequences of off-target activities that are elucidated in the in vitro screens. Also, the in vitro data can be invaluable in interpreting findings from the in vivo studies.

Dose selection for in vivo studies was somewhat controversial and engendered extensive discussion by the EWG. Because the purpose of these studies is to capture clinically relevant findings, therapeutic and supratherapeutic doses were considered to be necessary for inclusion. Acute toxicology studies served to guide dose selection for the safety pharmacology studies. Indeed, the final guideline incorporated the following change from step 2: "The guidance recommends that in the absence of a safety pharmacology response, the highest dose tested should be a dose associated with moderate toxicity. The guidance recommended that the highest dose tested should equal or exceed those doses producing some adverse effects."

The potential for significant adverse effects on various major organ systems was discussed by the EWG. It was agreed that adverse effects on cardiovascular, respiratory, and central nervous system (CNS) carried the greatest risk for catastrophic safety consequences and therefore are included in the core battery. Evaluation of safety pharmacology effects on other organ systems such as renal,

gastrointestinal, and autonomic nervous system is described under supplemental safety pharmacology studies with the recommendation that evaluating these organ systems should be considered when there is a cause for concern. Some sponsors routinely evaluate all of these organ systems in their safety pharmacology package, despite this not being a recommendation in the guideline. The guideline also suggests consideration of whether there is sufficient information available from toxicology studies to support safety in humans. The EWG expects that the sponsor will design the safety pharmacology evaluations in view of all of the information available for the test compound.

### *11.3.1 Central Nervous System*

The functional observation battery (FOB) (Mattsson et al. 1996) and modified Irwin's Test (Irwin 1968) in mice have a long history of use in evaluating safety of chemicals. The EWG determined that these assays are appropriate for detecting significant, pharmacologically mediated changes in motor activity, behavior, coordination, sensory/motor reflex responses, and body temperature in a standard, straightforward manner with a minimum of resources. This assay is included in the MHLW General Pharmacology Guideline, and the history of use of these assays in the chemistry industry provides a comforting database. By performing the assay under GLP, it is expected that persons conducting the assay be adequately trained and results with test substances are compared to positive and negative controls. Examples of more detailed CNS evaluations are mentioned in the ICH S7A guideline as follow-up assays. Drug dependence liability assessments are sometimes considered in the safety pharmacology scope, but are not discussed in ICH S7A because they are not acutely life threatening and therefore not needed to support the early clinical studies.

### *11.3.2 Cardiovascular System*

Adverse effects on the cardiovascular system are one of the most common reasons for discontinuation of development of promising drug candidates (Laverty et al. 2011) and have potential for severe adverse consequences. The EWG recommended that changes in heart rate, blood pressure, and electrocardiogram (ECG) be evaluated in the core battery cardiovascular assay and other indices, such as cardiac output, cardiac contractility, and peripheral vascular resistance, be considered in the follow-up assays. The reasoning was that significant (major) changes in cardiac and vascular function would be reflected in the endpoints in the core battery. It was recognized that there could be small effects on cardiac function or vascular resistance that will not be reflected in blood pressure and heart rate signals. However, if the magnitude of change in these parameters at dose levels many multiples over therapeutic levels is small, the safety risk in the clinic will be minimal. It is also recognized that functional cardiovascular effects are routinely and easily assessed in the clinical safety studies (phase 1).

At the time that ICH S7A was being discussed, no scientific consensus existed on the preferred approach to addressing risks for repolarization-associated ventricular tachyarrhythmia (i.e., Torsade de Pointes). Additionally there was no internationally recognized guidance on this topic. The EWG determined that this topic would be best served by a separate guideline that could bring the latest evolving information together (see Sect. 11.4).

### *11.3.3 Respiratory System*

Respiratory distress and acute bronchoconstriction are major clinical adverse events with potentially life-threatening consequences. Prior to ICH S7A, respiratory function was generally assessed in nonclinical pharmacology and toxicology studies via observation of depth and pattern of breathing. The EWG initially concluded that this was adequate; however, a case was made for more quantitative indices of respiratory function to support the safety of new drug candidates. The EWG concluded that more quantitative indices of respiratory function better supported the safety of new drug candidates. Therefore, the following change was incorporated following step 2: "The guidance recommends that, in addition to respiratory rate, other measures of respiratory function (e.g., tidal volume or hemoglobin oxygen saturation) should be evaluated in assessing effects of the test substance on the respiratory system."

### *11.3.4 Supplemental Safety Pharmacology Studies*

Studies to evaluate safety pharmacology effects in other organ systems are listed as supplemental studies. This is included in the ICH S7A guideline for sponsors to consider whenever there are potential safety concerns in other organ systems that are not evaluated in the core battery or other toxicology studies. As mentioned above, many sponsors have included other organ systems in their safety pharmacology packages.

## 11.4 S7B Guideline "Nonclinical Evaluation of the Potential for Delayed Ventricular Repolarization (QT Interval Prolongation) by Human Pharmaceuticals"

### *11.4.1 Background and Objectives*

Several drugs were removed from the market when it was recognized that they were associated with deaths due to a ventricular tachycardia called Torsade de Pointes (TdP). All of these drugs delayed ventricular repolarization (prolonged the QT interval of the surface ECG) via inhibition of a delayed rectifier potassium channel, $I_{Kr}$ (Darpo 2001; Redfern et al. 2003). $I_{Kr}$ is commonly referred to as the hERG channel.

The human Ether-à-go-go-Related Gene (hERG) is responsible for expression of the $I_{Kr}$ channel protein, and hERG is used to express the human protein in cell lines for $I_{Kr}$ bioassays. Identification of a molecular mechanism ($I_{Kr}$ inhibition) that contributes to risk of TdP and availability of an accessible index of delayed ventricular repolarization (QT interval prolongation on the surface ECG) in animals and humans provides opportunity to evaluate the risk for this type of cardiac activity during drug development.

In 1997, "Points to Consider: The Assessment of the Potential for QT Interval Prolongation by Non-Cardiovascular Medicinal Products" (CPMP/986/96) was issued by the Committee for Proprietary Medicinal Products (CPMP) (Anon 1997). This was the first regulatory document to describe a nonclinical testing strategy for assessing risk of QT interval prolongation as a means to reduce the risk of drug-induced TdP. The document recommended measurement of action potential duration (APD) in in vitro cardiac preparations (e.g., rabbit Purkinje fiber) and changes in ECG QT interval duration in in vivo animal models. While the scientific rationale for employing these two assays to assess risk for a drug to prolong ventricular repolarization (increases in APD at the cardiac cellular level and QT interval at the surface ECG) was sound, there were questions about the reliability of these assays and how to use these data for risk assessments in humans. Also, at the time of these recommendations, the role of $I_{Kr}$ inhibition as a common molecular mechanism for drug-induced prolongation of ventricular repolarization was not known.

With this background, the task for the ICH S7B EWG was to develop a guideline using the CPMP document, as well as a draft guidance from Health Canada (Strnadova 2005) as starting points. Outstanding issues to be addressed by the EWG included translation of $I_{Kr}$ inhibitory potencies to risk of QT interval prolongation, accurate measurement of QT interval duration as a reliable index of changes in ventricular repolarization, relationship between QT interval prolongation, and TdP. It was first determined that a guideline could provide value by recommending a testing strategy to assess the risk of delayed ventricular repolarization (QT interval prolongation), but it was unrealistic at that time to develop guidelines for assessing the risk for drug-induced TdP arrhythmia. Therefore, the title and objective of ICH S7B refer to assessing the risk of delayed ventricular repolarization and not the proarrhythmia risk for drug candidates.

It is important to note that the safety concern was an unexpected cardiac toxicity associated with mortality, which had occurred with several noncardiac drugs. While the incidence of toxicity was low, some of the drugs such as the antihistamine, terfenadine (Seldane®), were widely prescribed; therefore, the risk was considered unacceptable when considered over the population of users at large. Recognition of the relationship between delayed ventricular repolarization and risk for TdP was confirmed from investigation of the genetic QT prolongation syndrome as well as drug-induced QT interval prolongation. In both of these scenarios, the QT interval prolongation was only one of several risk factors that needed to be present at the same time to induce the arrhythmia and hence the very low incidence of arrhythmias even when the QT interval is delayed. Again, the objective of S7B is to assess the risk for delayed ventricular repolarization by drugs as a strategy to reduce one of the risk factors for TdP. Because of the low incidence of TdP, determining if the strategy

reduces the risk of TdP requires very large patient experience (i.e., absence of TdP in clinical trials prior to registration is usually not sufficient to exclude this risk).

After the ICH S7B EWG was underway, developing a guideline for the clinical assessment risk of QT interval prolongation becomes an ICH topic. The ICH E14 EWG began working on their guidance, "Clinical Evaluation of QT/QTc Interval Prolongation and Proarrhythmic Potential for Non-Antiarrhythmic Drugs." During this time, there were joint EWG meetings so that the two guidelines could be aligned, and it was agreed to publish both at the same time. A controversial issue was the need for clinical assessment of QT interval prolongation risk when the nonclinical assessment indicated there was very low risk. At the time of introduction of the guidelines, there was no prospective experience to conclude the clinical studies were not needed; however, this has been an ongoing discussion and a topic of great interest to all involved (Trepakova et al. 2009).

### *11.4.2 Nonclinical Studies Performed to Support ICH S7B*

The interest in achieving a practical and effective guideline was shared by the pharmaceutical industry, academia, and regulatory agencies. As a result there were several studies performed (and published) that provided useful information for the ICH S7B EWG. One was an investigation performed under the guidance of the ILSI-HESI Cardiovascular Safety Subcommittee where positive and negative control drugs (all with clinical experience) were prospectively tested in three nonclinical assays: inhibition of $I_{Kr}$ in vitro, APD prolongation in vitro (canine Purkinje fiber), and QT interval prolongation in vivo (conscious dogs instrumented with telemetry). The results (Hanson et al. 2006) demonstrated that (1) the $I_{Kr}$ inhibitory potency was reliably measured in two independent laboratories using cells lines with hERG expression of $I_{Kr}$, (2) the canine Purkinje fiber APD assay had a significant number of false negatives, and (3) the in vivo assay correctly identified all drugs with QT interval prolonging activity. Members of Japanese Pharmaceutical Manufacturers Association (JPMA) also did a series of prospective studies (Ando et al. 2005; Hayashi et al. 2005; Kii et al. 2005; Miyazaki et al. 2005; Omata et al. 2005; Sasaki et al. 2005; Tashibu et al. 2005; Toyoshima et al. 2005; Yamazaki et al. 2005) expanding the findings of the ILSI-HESI group to the guinea pig papillary muscle for APD assessment and nonhuman primate for in vivo QT assessment. The Association of the British Pharmaceutical Industry (ABPI) group lead by Tim Hammond performed a retrospective study to determine acceptable safety margins for potency at the $I_{Kr}$ channel (Redfern et al. 2003). Their conclusion was that when the margins (adjusted for plasma protein binding) are greater than 30-fold, the risk for QT interval prolongation is low. This of course is a very broad generalization, but does support the concept that margins should be considered and not all inhibitors of $I_{Kr}$ have the same risk for adverse CV effects. The ICH S7B EWG was fortunate to have these prospective and retrospective data, as well as scientific input from scientists in all three ICH regions.

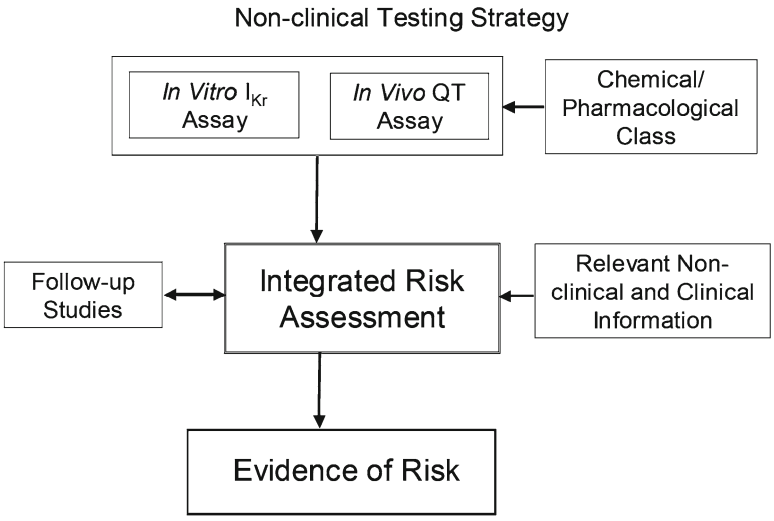


**Fig. 11.1** Nonclinical testing strategy from ICH S7B guideline

### *11.4.3 Testing Strategy and Assay Selection*

The ICH S7B EWG created Fig. 11.1 to illustrate the general testing strategy. The EWG recommends that the sponsor consider whether the test substance belongs to a pharmacological or chemical class that is associated with a known risk for QT interval prolongation and/or TdP. For example, many antipsychotic and non-sedating antihistamine drugs have been associated with QT interval prolongation and TdP in humans. In such cases, the sponsor is encouraged to pursue testing that strategy that directly compares the test compound to those in the same class with documented risk.

Following the precedent to recommend a combination of core battery, follow-up, and supplementary assays in ICH S7A (see above), the ICH S7B EWG spent significant time debating the assays that should be the core battery assays.

Because all of the drugs removed from the market due to an association with TdP delayed ventricular repolarization by inhibiting $I_{Kr}$ and because the human form of the channel protein can be expressed in cell lines, an In Vitro Ion Channel Assay was included in the core battery (see section "In Vitro Ion Channel Assay").

Testing for the potential for drug candidates to prolong cardiac APD is recommended in the CPMP Points to Consider document (see Sect. 11.4.1) and is a logical step to determine if inhibition of $I_{Kr}$ detected in the In Vitro Ion Channel Assay translates into APD prolongation in a multicellular preparation. However, based upon EWG experience, as well as the results from the ILSI-HESI study (Hanson et al. 2006), there was concern about the high incidence of false-negative results in the Purkinje fiber APD assay. It was recognized that when activity is detected in in vitro APD assays, the results can be very important in characterizing the relative

risk and potential effects of the test compound on other APD parameters, including other cardiac ion channels. As a result, the APD assay was included as a follow-up assay (see section "Follow Up Assays"). Other in vitro assays such as the rabbit Langendorff heart (Hondeghem et al. 2001; Hondeghem 2006) and the ventricular wedge preparation (Yan and Antzelevitch 1996; Liu et al. 2006) that measure additional characteristics of repolarization (e.g., instability of APD changes and dispersion of refractoriness, respectively) were discussed and included in the supplementary assays due to their technical complexity and their focus on arrhythmia risk rather than simply duration of ventricular repolarization.

An in vivo assay directly measuring QT interval duration was included in the core battery because it has the potential to detect effects of drug candidates on ventricular repolarization by any mechanism or combination of mechanisms. As shown in Fig. 11.1, the In Vivo QT Assay is the final step in the core battery because it integrates a drug's effects on ventricular repolarization, and is analogous to the clinical setting (including the bioassay recommended in ICH E14) to assess risk of QT interval prolongation in humans (see section "In Vivo QT Assay").

#### 11.4.3.1 In Vitro Ion Channel Assay

All drugs that have a direct inhibitory effect on $I_{Kr}$ will delay ventricular repolarization in vivo when appropriate plasma concentrations are achieved in the heart, and there are no other electrophysiological effects that modulate the effects of $I_{Kr}$ on ventricular repolarization. The basic pharmacological principle is that inhibition with selective $I_{Kr}$ blockers is concentration related and relative potency data can be used to compare compounds and estimate safety margins, as was shown by Redfern et al. (2003). The In Vitro Ion Channel Assay uses the human protein; however, because the structure and therefore pharmacology of $I_{Kr}$ are similar across species, translation of relative potencies at the $I_{Kr}$ channel level—from in vitro human to in vivo dog, nonhuman primate or swine—is very good, and translation is acceptable from nonclinical in vivo to clinical settings.

It is important to recognize that translation of in vitro potency into in vivo activity is influenced by factors that affect access of the test compound to the $I_{Kr}$ ion channel, such as metabolism, distribution, and plasma protein binding. Also, when the test compound has effects on multiple cardiac ion channels, estimating safety margins from in vitro $I_{Kr}$ inhibitory potencies alone is difficult. Therefore, not all $I_{Kr}$ blockers will prolong the QT interval in vivo at exposure levels where in vitro activity was observed. Note that ICH S7B does not make recommendations about the appropriate safety margin for the test substance because factors such as therapeutic indication (benefit–risk assessment), disposition, and other pharmacological characteristics (safety margin) should be considered by the sponsor. Also because of the complexity in predicting relative potencies in vivo, the in vitro potency values and safety margins are refined when in vivo data are available.

At the time of development of ICH S7B, the in vitro assay for assessing relative potency of $I_{Kr}$ inhibition used standard voltage clamp methodology. This is a

technically challenging assay. The EWG recognized that ligand binding assays were available, but, based upon the low specific activity of most the available radioligands, results are generally not robust enough for risk assessment. Since publication of ICH S7B, high-throughput voltage clamp assays have become available and can be adequate for the In Vitro Ion Channel Assay if sensitivity and specificity are defined. A practical problem with some of these new assay systems is binding of lipophilic compounds to the plastic in the high-throughput instruments, which may underestimate the inhibitory potency.

From clinical experience with drugs and information from congenital long QT syndrome, the ICH S7B EWG was aware that there are cardiac ion channel mechanisms in addition to $I_{Kr}$ inhibition that can delay ventricular repolarization in a manner that are risk factors for TdP. These include inhibition of $I_{Ks}$, agonism of the window sodium channel, and modulation of cardiac calcium channels. There is clear value in assessing the relative potencies of test substance on these other mechanisms early in the evaluation process (Hancox et al. 2008); however, the EWG concluded, because of the promiscuous behavior of the $I_{Kr}$ channel for inhibition by drugs (Sanguinetti and Mitcheson 2005; Sanguinetti and Tristani-Firouzi 2006), that this was the mechanism of greatest risk. It was also reasoned that if the other, less commonly seen mechanism(s) were present, they would be detected in the In Vivo QT Assay. In fact, if QT interval prolongation is observed in the In Vivo QT Assay that is inconsistent with the test substances inhibitory potency on $I_{Kr}$, the use of follow-up assays to explore the effects on other ion channels is prudent. Therefore, the decision to screen for mechanisms in addition to $I_{Kr}$ inhibition is left up to the sponsor, dependent on their risk tolerance for a possible non-$I_{Kr}$ mechanism QT interval prolongation appearing in the In Vivo QT Assay.

Since the publishing of ICH S7B, there is evidence that drugs can interfere with the "trafficking" of the $I_{Kr}$ channel protein to the surface of the cell. This is a potential mechanism for drugs to prolong the QT interval without directly inhibiting the $I_{Kr}$ channel (Delisle et al. 2004; Hancox and Mitcheson 2006). The turnover rates of the $I_{Kr}$ proteins or pharmacodynamic relationship between level of inhibition and delay in repolarization are not known, making it challenging to interpret the relative risk of QT interval prolongation from the available in vitro trafficking assays. The ICH S7B EWG did not discuss this topic, so this mechanism is not included in the guideline.

#### 11.4.3.2 In Vivo QT Assay

QT interval prolongation of the electrocardiogram (ECG) is a consequence of APD prolongation at the cellular level and delayed ventricular repolarization at the organ level. Therefore, measuring the QT interval duration in relevant animal models and in humans is a practical approach to assessing delay in ventricular repolarization. As such, the In Vivo QT Assay is a central component of the S7B testing strategy and relates directly to objectives and endpoints in ICH E14 and other clinical safety testing.

The cardiovascular assays in the ICH S7A core battery and the GLP toxicity studies include evaluation of the ECG; however, it was recognized that additional considerations are required to evaluate the risk of drug-induced delayed ventricular repolarization. This was an important topic for the ICH S7B EWG in developing the guidance. First, the species used to assess risk of QT interval prolongation in humans needs to be considered. Unlike humans, the duration of ventricular repolarization is not controlled by $I_{Kr}$ in rodents, and therefore, one cannot assess the risk of QT interval prolongation for humans using rats or mice. In the ICH S7A guidance, there is no recommendation for species in the cardiovascular assessment (see Sect. 11.3.2). If the sponsor chooses to use rodents for the ICH S7A assessment, an additional study in non-rodents is needed to investigate the effects on ventricular repolarization and comply with recommendations in ICH S7B. Second, the sensitivity of ECG recordings to detect changes in QT interval is rather poor in toxicology studies due to high sympathetic tone and variable heart rates with methods of restraint and brief sampling periods. The availability of implantable telemetry devices for dogs and nonhuman primates as well as computer-assessed measurement of ECG intervals provided an opportunity to capture high-quality ECG signals and evaluate many complexes over a long period. The ICH S7B guideline does not specifically recommend the use of telemetry but does recommend determining sensitivity and specificity of the assay/method used to support the risk assessment. Since finalization of ICH S7B, there are now alternatives such as jackets that can capture ECG data with reasonable quality without surgical implantation of a device (Chui et al. 2009; Kyle et al. 2009). Both ICH S7A and S7B describe an option to collect ECG data for QT intervals in the toxicology studies with the premise that sensitivity and specificity need to be defined in order to support conclusions from the data. Note that the level of sensitivity for detecting QT intervals is not dictated in the guideline, but the suggestion is that the sponsors use an assay that has sensitivity appropriate for the risk. It has been challenged whether toxicology studies can adequately assess risk of drug-induced QT interval changes; however, in principle, QT interval data from toxicology studies will be in compliance with ICH S7B if guideline recommendations concerning sensitivity are satisfied (see Guth et al. 2009).

Measurement of changes in QT interval duration as an index of ventricular repolarization is not straightforward. The duration of the QT interval is significantly affected by changes in the heart rate, respiratory patterns, and autonomic nervous system activity. The ICH S7B EWG discussed the value of assessing changes in QT interval in anesthetized preparations where some of these variables can be controlled; however, the consensus was that the conscious, unrestrained animal would be the more appropriate setting for predicting risk in humans. It is recommended that sponsor consider using the anesthetized preparation when there are drug-induced changes in sympathetic tone or as a follow-up assay to determine if changes detected in the conscious preparation are direct effects on ventricular repolarization or a consequence of altered autonomic tone and/or overcorrection with QT interval heart rate correction formulae.

There is no absolutely reliable method to adjust QT interval duration measurements for changes in heart rate or autonomic tone. There are several correction formulae (Miyazaki and Tagawa 2002) which are typically valid over small changes in heart rate. Given this dilemma, ICH S7B makes no recommendations beyond justifying the choice of heart rate correction formula with data from the test system. The guideline

also recommends that sponsors consider analyzing the data by plotting the QT/RR relationship. Heart rate correction of QT intervals is also an issue for the corresponding clinical assay in ICH E14. In many cases, sponsors will analyze the data with several formulae and discuss the totality of data set to support their conclusions.

Species differences in potencies for QT interval prolongation in vivo are not due to species differences at the channel level (i.e., relative potency for $I_{Kr}$ inhibition; see section "In Vitro Ion Channel Assay"). They can be due to differences in distribution, metabolism, plasma protein binding, background autonomic tone (including baseline heart rate), and other cardiovascular effects. Therefore, the guideline makes no specific recommendations as to a preferred species for this assay but does recommend that the sponsor select and justify the most appropriate in vivo test systems and species.

#### 11.4.3.3 Follow-Up Assays

As discussed above (Sect. 11.2.1), the objective of follow-up assays is to obtain additional information to interpret and/or provide context for results from assays in the safety pharmacology core battery, pharmacology and toxicology studies, and clinical studies.

For example, when results from the In Vitro Ion Channel and In Vivo QT Assays are not consistent with one another, there are several options for follow-up assays. When there is in vivo but not in vitro activity, testing metabolites for $I_{Kr}$ inhibitory potencies is prudent. If the test compound is active in an in vitro APD assay (Purkinje fiber assay, Langendorff heart preparation, or ventricular wedge assay), assessment of configuration of APD prolongation (APD 30 vs. APD 90) can be helpful in evaluating the consequences of multiple ion channel activities. As mentioned above (Sect. 11.4.1), if the test compound does not prolong the APD (but does prolong QT interval in vivo), results from the APD assay will not be useful. Another follow-up strategy is to test the potencies on other cardiac ion channels. To determine if the heart rate correction formulae might be overcorrecting the duration of the QT interval, a beat-to-beat analysis of the relationship between heart rate and QT duration may be helpful (Fossa et al. 2005).

When the risk of QT interval prolongation is defined for a test compound, follow-up assays are sometimes employed to determine if the proarrhythmic risk is consistent or less than expected from the change in repolarization (see Sect. 11.5).

#### 11.4.3.4 Assays/Strategies for Assessing Proarrhythmia Risk

Assessment of the safety of drug candidates in simulated pathological conditions and arrhythmias is very challenging because of the abundant combinations of risk factors in the broad patient populations. The ICH S7B EWG did not provide specific guidance on this, but provided the following statement in the document: "Interested parties are encouraged to develop these models and test their usefulness in predicting risk in humans."

QT interval prolongation is only one of the several risk factors that must be coincident to be a trigger for TdP (Kowey and Malik 2007); therefore, the incidence of TdP, even

when there is QT interval prolongation, is very small (Darpo 2001, 2007). Because the combinations of risk factors are many and the incidence of TdP is so low, one cannot typically exclude risk of TdP from data in a typical clinical development program. Unless there is a high incidence of TdP, exclusion of risk will usually require post-marketing data. Therefore, when there is a risk for QT interval prolongation at or near therapeutic levels, the label of an approved drug will carry a warning of potential risk for TdP. In this case, prior to approval to market, the sponsor may want to determine how this risk relates to other drugs in the class with and without a significant risk of TdP.

There have been at least two symposia addressing this issue, one by the European Society of Cardiology (Haverkamp et al. 2000) and one by ILSI-HESI (Bass et al. 2004). In both symposia, measurable attributes of test substances that might signal increased risk of TdP were discussed, including dispersion of refractoriness, instability of repolarization, and changes in action potential configuration. No single assay has been proposed, and the prediction is that a battery of nonclinical assays will be needed. There is a case study since launch of ICH S7B where the sponsor successfully made the case for a low risk of TdP despite a clear risk of QT interval prolongation. This case is ranolazine where both nonclinical and clinical data were used. The combination of pharmacological activities of ranolazine on $I_{Kr}$ and $I_{Na}$ was shown to (1) prevent the expected APD prolongation and incidence of early after depolarizations with a potent $I_{Kr}$ blocker, (2) have less than expected transmural dispersion of refractoriness compared to $I_{Kr}$ blocking drugs with a history of TdP in a ventricular wedge preparation (Antzelevitch et al. 2004), and (3) exhibit a decline in incidence of ventricular tachycardia in patients with non-ST segment elevation acute coronary syndrome (Schram et al. 2004; Song et al. 2004). Therefore, demonstrating a lower than expected risk for TdP with a drug that blocks $I_{Kr}$ and prolongs the QT interval prolongation requires a well-designed strategy with use of positive and negative reference agents. There are likely to be regional differences in how regulators interpret these data.

### *11.4.4 Integrated Risk Assessment and Evidence of Risk*

The ICH S7B guideline recommends that an Integrated Risk Assessment for QT interval prolongation be used to maximize the value of the experimental data by considering all of the available information including the targeted indication and patient population. Information on the sensitivities of the nonclinical assay used, relative potencies of the test compound compared to reference drugs, characteristics of the primary pharmacology that could impact risk for QT interval prolongation, and risk of greater exposure due to hepatic impairment or drug–drug interactions are important components. The Integrated Risk Assessment is an important opportunity for the sponsor to make a scientific case that either the risk of QT interval prolongation with their development candidate is negligible at therapeutic levels or the benefit/risk assessment is acceptable for the intended use and indication(s). The objective of generating an Integrated Risk Assessment is to enable prudent decisions by sponsors and regulators, as well as to provide information that can be used to help describe nonclinical data in future labels. The Integrated Risk Assessment should be updated as additional data become available,

including clinical data. The guideline recommends that the Integrated Risk Assessment be included in the Investigator's Brochure and the Nonclinical Overview (ICH M4). Including an Integrated Risk Assessment in regulatory documents is a valuable opportunity for sponsors to insure their data are presented in the most effective way.

The concept of evidence of risk was included in ICH S7B to emphasize that the evaluation of risk is not an all-or-nothing proposition. Initially, it was the intention of the EWG to provide a qualitative scale for ranking and communicating the relative risk for the test compound to prolong the QT interval. This turned out to be too ambitious given the complexity and spectrum of data and indications. Describing evidence of risk in a very qualitative manner in ICH S7B was done to encourage sponsors to provide a context for risk in the Integrated Risk Assessment.

### *11.4.5 Relationship Between ICH S7B and ICH E14 Guidelines*

Ideally, the nonclinical and clinical guidelines should be complementary, and the results from the studies recommended by both are to be used in the risk assessment. The ICH S7B EWG recommended that conclusions from the nonclinical studies would contribute to the design and interpretation of the clinical studies assessing risk of QT interval prolongation. For example, when no risk is identified in the ICH S7B studies, the need for a thorough clinical QT/QTc study would be reduced. Also, "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" (text from ICH S7B). Analyses of both nonclinical and clinical data would be important to avoid false-positive and false-negative outcomes from either nonclinical or clinical studies. The ICH E14 EWG was not confident that the nonclinical study results would be predictive of the clinical situation and, at the time, there were no prospective data to address this concern. Therefore, at the time of implementation of the guidelines, the results of the clinical assessment alone were deemed the final arbiter of risk for QT interval prolongation in humans. More recently, ILSI-HESI has a project to investigate the concordance among nonclinical and clinical studies as well as the need for a thorough clinical QT/QTc study when no risk is identified in nonclinical studies (Trepakova et al. 2009).

## 11.5 Post-S7A and Post-S7B Implementation: Lessons Learned and Future Opportunities

Safety pharmacology studies are currently performed by pharmaceutical companies and contract research organizations and have been successfully integrated into preclinical drug development programs (Ewart et al. 2012). Most regulatory filings include data from the assays in the core batteries recommended in ICH S7A and

S7B, and there are usually minimal or no data from the supplementary and follow-up assays. The Safety Pharmacology Society (http://www.safetypharmacology.org) has become a valuable venue for sharing experiences and advancing new ideas and technologies in safety pharmacology (Redfern and Valentin 2011; Cavero 2011).

The recommendations in the guidelines have prompted development of technologies to capture safety pharmacology data such as whole-body plethysmography for assessing indices of respiratory function and implantable/wearable telemetry devices for capturing cardiovascular endpoints in conscious, unrestrained subjects. While this has standardized the assay methodology to a certain degree, there has been a focus on data collection more than interpretation of data and translation to risk.

It would be valuable to evaluate retrospectively the benefit and cost of the ICH S7A- and S7B-recommended studies. Specifically, have these studies effectively reduced attrition of drug candidates? Have they improved the safety of clinical trial participants? Are the resources used to perform these studies in development (i.e., GLP), including use of animals, justified compared to assessing off-target liabilities during lead optimization (see Cavero 2009)?

## 11.6 Conclusions

The key objectives of the safety pharmacology guidelines are to encourage sponsors to use testing strategies based upon a scientific rationale appropriate for drug candidate, to provide flexibility, and to support interpretation of results in a scientific manner. Such strategies involve validation of assays, definition of sensitivity and specificity, and comparison of results with positive and negative reference drugs (with clinical experience). The recommendations in the guidelines are intended to encourage sponsors to use an evidence-based risk assessment for their compound to support safety for clinical trial participants and patients as well as to reduce attrition of drugs in clinical development.

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