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

Training Materials

March 2022

International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use

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Background

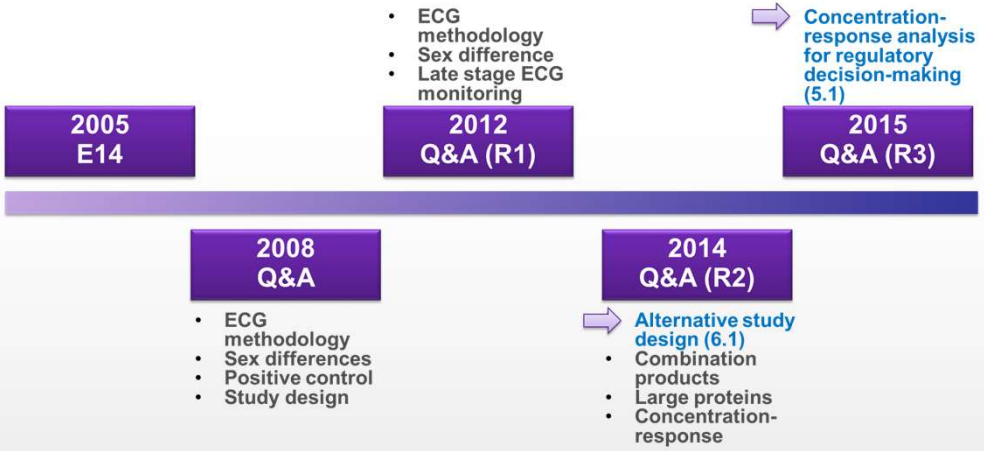
- **This document has been signed off as *Step 4* document (21 February 2022) to be implemented by the ICH Regulatory Members**
- **This document was developed based on a Concept Paper (15 November 2018)**
- **This online training slide presentation**
  - Summarizes the updates made to the E14 Q&As and the new Q&As to S7B
  - Provides rationale for some of the recommendations in the Q&As
  - Contains examples of how to implement the Q&As
- **It is anticipated the additional training material will be made available subsequently**

Key Principles – ICH S7B Guideline

- ICH S7B ("The Non-Clinical Evaluation of the Potential for Delayed Ventricular Repolarization [QT Interval Prolongation] by Human Pharmaceuticals") was established in 2005
- A mechanistic understanding of the development of torsade de pointes (TdP) and the emergence of new types of assays have made it possible to obtain more information to assess TdP risk from nonclinical assays
- The *in vitro* IKr/hERG assay and *in vivo* QT assay as well as optional follow-up studies, as described by the ICH S7B guideline, are conducted for hazard identification and risk assessment relevant to delayed ventricular repolarization.
- In addition to supporting the planning and interpretation of First-in-Human clinical studies, nonclinical investigations can also contribute to an integrated risk assessment for TdP in later stages of development when clinical data are available

Key Principles – ICH E14 Guideline

- ICH E14 “The Clinical Evaluation of QT/QTc Interval Prolongation and Proarrhythmic Potential for Non-Antiarrhythmic Drugs” was established in 2005
- It provides recommendations concerning the design, conduct, analysis, and interpretation of clinical studies to assess the potential of a drug to delay cardiac repolarization
- Multiple prior Q&As have been developed since 2005



Key Principles – Integrating ICH E14 and S7B

- As implemented to date, S7B studies have primarily informed safety before first-in-human dosing but then generally have not been considered in regulatory decision-making once drugs enter clinical development
- As both the ICH S7B and E14 guidelines highlight the need for integration of information in a manner which is informative as a totality of evidence …
- … these Q&As focus on connecting the ICH S7B and E14 Guidelines for scenarios where the nonclinical data are informative in clinical study implementation and evaluation

Key Principles – The Revised and New Q&As

- **E14 Q&As (R3) were finalized in 2005 and with 7 Q&As**
- **The new E14 Q&As contain revisions to Q&As 5 and 6 only**
  - E14 Q&A 5 - Use of Concentration Response Modeling of QTc Data
  - E14 Q&A 6 - Special Cases (updates to Q&A 6.1 only)

- **S7B did not have any prior Q&As and now contains 4 Q&As**
  - S7B Q&A 1 - Integrated Risk Assessment
  - S7B Q&A 2 - Best Practice Considerations for *In vitro* Studies
  - S7B Q&A 3 - Best Practice Considerations for *In vivo* Studies
  - S7B Q&A 4 - Principles for Proarrhythmia Models

Q&As Objectives & Scope

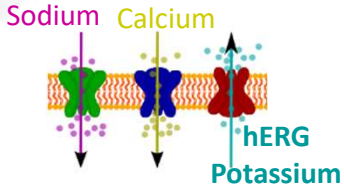
E14 and S7B Q&As

How to apply the integrated risk assessment in clinical development

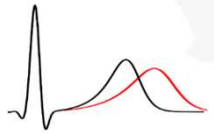
- To decrease the need for clinical Thorough QT (TQT) studies (E14 Q&A 5.1)
- To inform regulatory decision making when a TQT (or equivalent) can't be performed (E14 Q&A 6.1)

S7B Q&As on Best Practices

Ion channels



In vivo QT



Cardiomyocytes

Proarrhythmia models

Figure modified from Strauss et al. *Clinical Pharmacology & Therapeutics*, 2021

Q&As Detailed Summary

a) **Diagram from the original S7B Guideline.**

b) **New S7B Q&As on best practice considerations for the core S7B assays (hERG and *in vivo* QTc) and additional ion channel assays that can be used as follow-up studies.**

c) **New S7B Q&As on best practice considerations for *in vitro* cardiomyocyte assays and principles for proarrhythmia models.**

d) **The new S7B integrated risk assessment Q&As in combination with the revised E14 Q&As describe how nonclinical data can be used to reduce the number of TQT studies and reach a low-risk determination when a TQT or equivalent cannot be performed. The integrated risk assessment also describes how follow-up studies can be used to understand and predict TdP risk of QTc-prolonging drugs, however, these are evaluated on a case-by-case basis.**

ECG, electrocardiogram; ICH, International Council for Harmonisation; Q&A, question and answer; QTc, heart rate corrected QT interval; TdP, torsade de pointes; TQT, thorough QT.

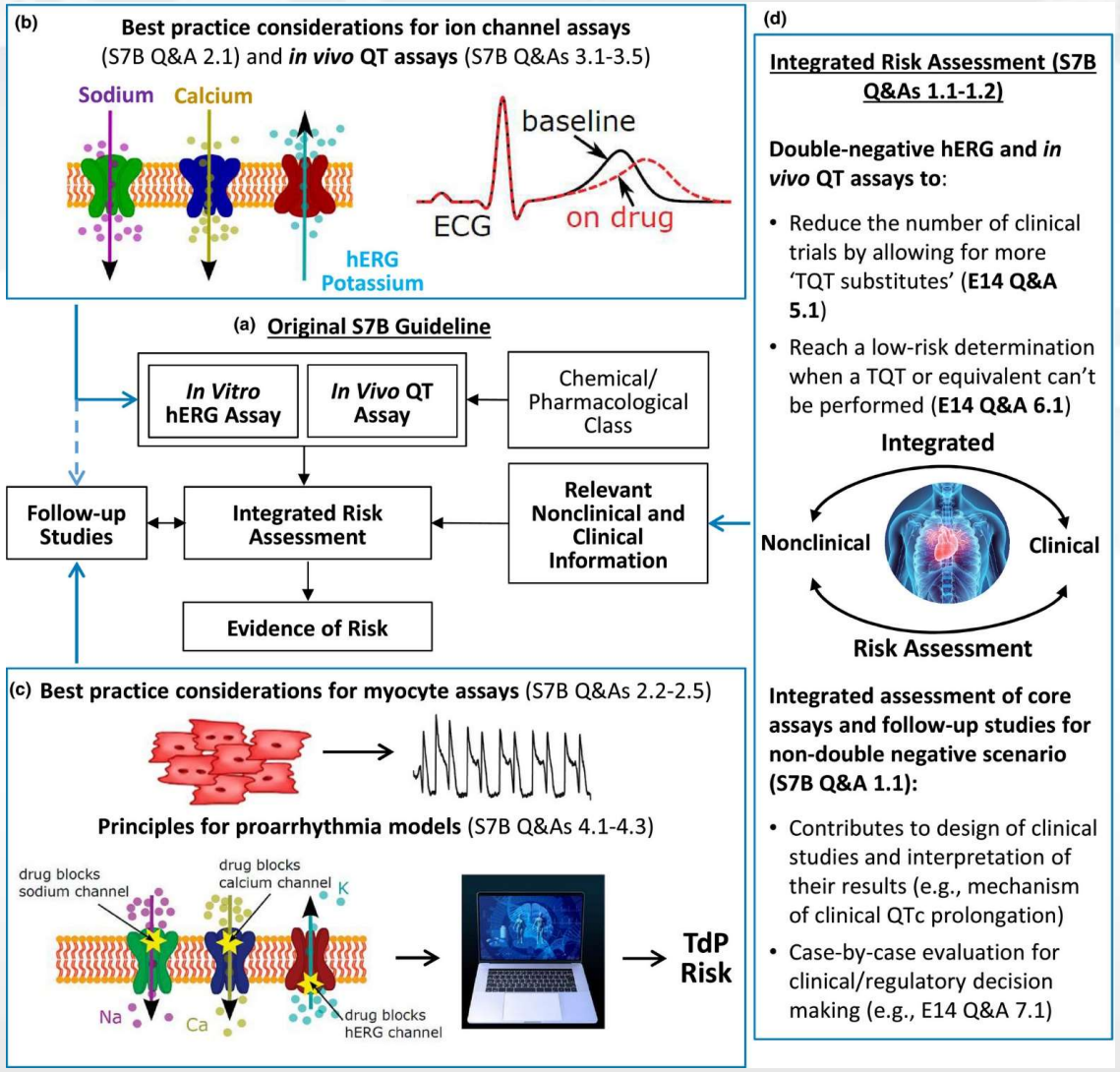


Figure reproduced from Strauss et al. *Clinical Pharmacology & Therapeutics*, 2021

ICH E14 Q&As 5.1 and 6.1

- Q&A 5.1: The ICH E14 Guideline states (in Section 3.2.3, page 10) that analysis of the relationship between drug concentration and QT/QTc interval changes is under active investigation. Has this investigation yielded a reasonable approach to concentration-response modeling during drug development? How can assessment of the concentration-response relationship guide the interpretation of QTc data?
- Q&A 6.1 The ICH E14 Guideline states that in certain cases a conventional thorough QT study might not be feasible. In such cases what other methods should be used for evaluation of QT/QTc and proarrhythmic potential?

E14 Q&A 5.1 – Question and Revised Answer (1 of 3)

- **Question**: The ICH E14 Guideline states that analysis of the relationship between drug concentration and QT/QTc interval changes is under active investigation. Has this investigation yielded a reasonable approach to concentration-response modeling during drug development? How can assessment of the concentration-response relationship guide the interpretation of QTc data?
- **Updates in the Answer:** Some text updates throughout with two main changes:
  - Addition of a section on dose and exposure definitions that apply to this Q&A, and are also cross-referenced in other Q&As (including S7B Q&As)
  - Modification to consideration #4 in E14 Q&A
    - **Prior wording**: If there are data characterizing the response at a sufficiently high multiple of the clinically relevant exposure (see E14 Section 2.2.2), a separate positive control would not be necessary.
    - **New wording**: next slide

E14 Q&A 5.1 – Revised Answer (2 of 3)

Addition of dose and exposure definitions

- **Therapeutic dose**: dose evaluated in Phase 3 trial or recommended in product labeling
- **Clinical exposure**: mean steady state maximum concentration (Cmax,ss) associated with the maximum therapeutic dose
- **High clinical exposure**: exposure (Cmax,ss) achieved when the maximum therapeutic dose is administered in the presence of the intrinsic or extrinsic factor (e.g., organ impairment, drug-drug interaction, food effect, etc.) that has the largest effect on increasing Cmax,ss
- **Supratherapeutic dose**: dose that provides exposures (mean Cmax) exceeding the high clinical scenario

E14 Q&A 5.1 – Revised Answer (3 of 3)

4. A separate positive control would not be necessary if either of the following conditions is met:

a) There are data characterizing the response at a sufficient multiple (commonly 2x) of the high clinical exposure (see ICH E14 Section 2.2.2);

Or b) If the high clinical exposure has been achieved in the clinical ECG assessment, but a sufficient multiple has not been obtained (e.g., for reasons of safety or tolerability, saturating absorption, etc.), then a nonclinical integrated risk assessment can be used as supplementary evidence. The reason higher doses were not tested should be adequately justified.

- **See ICH S7B Q&A 1.1 for details; in summary, the nonclinical studies should include**
  - (1) a hERG assay, following best practice considerations (see ICH S7B Q&A 2), that shows low risk as defined in ICH S7B Q&As 1.1-1.2 and
  - (2) no evidence of QTc prolongation in an *in vivo* assay conducted according to ICH S7B at exposures that cover high clinical exposures (see ICH S7B Q&As 1.1 and 3; note that some recommendations only apply to decision making under ICH Q&A 6.1).

5.1 Example

- **Shows a data package for a hypothetical drug to support an integrated risk assessment**
- **Supporting tables:**
  - Table 1-A: Clinical QT Assessment
  - Table 1-B: *In vitro* hERG Assay Evaluation
    - Deviations from best practice
  - Table 1-C: *In vitro* Assay Results
    - Safety margin assessment using reference compounds
  - Table 1-D: *In Vivo* QT Assessment

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

See Training Material Examples Supplemental File (Example 1)

Table 1-C: Reuse safety margin from reference compounds

- Investigational drug and reference drugs are evaluated under the same experimental protocol (blue shaded cells)
- Concurrent positive control is 1 of the reference drugs used to derive the threshold (orange shaded cells)
- IC_{50} of positive control is similar to the expected range of IC_{50} under the same experimental protocol (yellow shaded cells)
- Directly compare the hERG safety margin of parent and metabolite to safety margin threshold (green shaded cells)
- Conclude that the Investigational drug has low QTc prolongation risk at high clinical exposures because the hERG safety margins for parent and metabolite are higher than the preestablished hERG safety margin threshold.

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

See Training Material Examples Supplemental File for additional details (Example 1, Table 1-C)

E14 Q&A 6.1 – Question and Revised Answer (1 of 3)

Question: The ICH E14 Guideline states that in certain cases a conventional thorough QT study might not be feasible. In such cases what other methods should be used for evaluation of QT/QTc and proarrhythmic potential?

Answer:

- An integrated nonclinical-clinical QT/QTc assessment can be particularly valuable when a TQT study or E14 Q&A 5.1 approach is not feasible
  - Can apply when placebo-controlled comparison is not possible; safety considerations preclude administering supratherapeutic doses to obtain high clinical exposures and/or safety or tolerability prohibit the use of the product in healthy participants
  - Also valuable for drugs with confounding heart rate effects that could impact accurate determination of the QTc

- The integrated nonclinical and clinical QT/QTc risk assessment should include:
  1. The hERG assay, an *in vivo* QT assay, and any follow-up nonclinical studies, especially those selected to overcome the challenges encountered in the clinical studies (see ICH S7B Q&As 1.1 and 1.2); and
  2. Alternative QT clinical study designs incorporating ECG assessments with as many of the usual “thorough QT/QTc” design features as possible (see ICH E14 Section 2.2 and Q&A 5.1).

E14 Q&A 6.1 – Revised Answer (2 of 3)

Decision Making:

- A totality of evidence argument based on the integrated nonclinical-clinical QT/QTc assessment could be made at the time of marketing application
- A drug that meets the following criteria would be considered to have a low likelihood of proarrhythmic effects due to delayed repolarization:
  1. Nonclinical studies, following best practice considerations for *in vitro* studies (see ICH S7B Q&A 2) and *in vivo* studies (see ICH S7B Q&A 3), show low risk as defined in ICH S7B Q&A 1.1
  2. High-quality ECG data do not suggest QT prolongation, generally defined under this Q&A as an upper bound of the two-sided 90% confidence interval around the estimated maximal effect on ΔQTc less than 10 ms
  3. Cardiovascular safety database that does not suggest increased rate of adverse events that signal potential for proarrhythmic effects (ICH E14 Section 4)

E14 Q&A 6.1 – Revised Answer (3 of 3)

Regarding the clinical QTc threshold:

- When justified, a totality of evidence argument for a low likelihood of proarrhythmic effects due to delayed repolarization could still be made for a drug that has an upper bound of the two-sided 90% confidence interval around the estimated maximal effect on ΔQTc of 10 ms or more
- The determination will depend on the quality and details of the clinical data (e.g., estimated ΔQTc mean and upper bound values, slope of any concentration-ΔQTc relationship) and nonclinical data (e.g., difference between the hERG safety margin for the investigational drug and the threshold for defining low risk)

If nonclinical studies do not show low risk (or are not performed), decision making reverts to how it was in E14 Q&A (R3):

- There is reluctance to conclude a lack of an effect in the absence of a positive control
- However, if the upper bound of the two-sided 90% confidence interval around the estimated maximal effect on ΔQTc is less than 10 ms, the treatment is unlikely to have an actual mean effect as large as 20 ms

6.1 Example

- **Show a data package for a hypothetical drug to support an integrated risk assessment for ICH E14 Q&A 6.1.**
- **Support tables:**
  - Table 2-A: Clinical QT Assessment
  - Table 2-B: *In vitro* hERG Assay Evaluation
  - Table 2-C: *In vitro* Assay Results
  - Table 2-D: *In vivo* QT Assessment
    - Sensitivity assessment using historical positive control

QT assessment pathway	☐ *Substitute for thorough QT study (5.1)* ☑ *Alternative QT study when a thorough QT study is not feasible (6.1)* ➢ *6.1 pathway is appropriate because doses higher than maximum tolerated dose cannot administered to obtain high clinical exposures and the tolerability prohibit the use of the product in healthy participants.*
Clinical QT study findings	*Therapeutic dose (250 mg QD): 3.3 (90% CI 2.0, 4.5) ms at mean $C_{max,ss}$ (145 ng/mL)* ➢ *Alternative QT clinical study designs should incorporate ECG assessments with as many of the usual "thorough QT/QTc" design features as possible (see Table 2-A).*
Clinical adverse events	*In the pooled database of active-controlled clinical trials, there are no reports of TdP, ventricular tachycardia, ventricular fibrillation or flutter, sudden death, syncope or seizures. None of the subjects reported QTc >500 ms or an increase from baseline QTc >60 ms.* ➢ *No increased rate of adverse events that signal potential for proarrhythmic effects (ICH E14 Section 4).*
In vitro findings	*Safety Margin* | *Reference Drug Safety Margin* | *Best Practice Deviations* *Parent* | *95x* | *51x* | *Met best practice* ➢ *A hERG safety margin was higher than the threshold defined based on the safety margins computed under the same experimental protocol for a series of drugs known to cause TdP (see Tables 2-B and 2-C)*
In vivo findings	*The minimal detectable difference (MDD) in the assay (10 ms) is similar to the reported MDD from historical positive control; therefore, the exposure ratio should be greater than or equal to 3x to have similar sensitivity to clinical QT study based on historical positive control data.* *No QTc prolongation was observed at doses 5.0x the high clinical exposures.* ➢ *The study at 5.0x exposure and MDD of 10 ms had sufficient sensitivity to detect a QTc prolongation effect of a magnitude similar to dedicated clinical QT studies (see Table 2-D).*
Conclusion	*The drug has low likelihood of proarrhythmic effects due to delayed repolarization.* *a. The nonclinical studies following best practice considerations for in vitro and in vivo studies showed low risk for QTc prolongation. There are no major metabolites.* *b. The high-quality ECG data collected in the alternative QT clinical assessment do not suggest QT prolongation, defined as an upper bound of the two-sided 90% confidence interval around the estimated maximal effect on ΔQTc less than 10 ms as computed by the concentration-response analysis.* *c. The cardiovascular safety database does not suggest increased rate of adverse events that signal potential for proarrhythmic effects.*

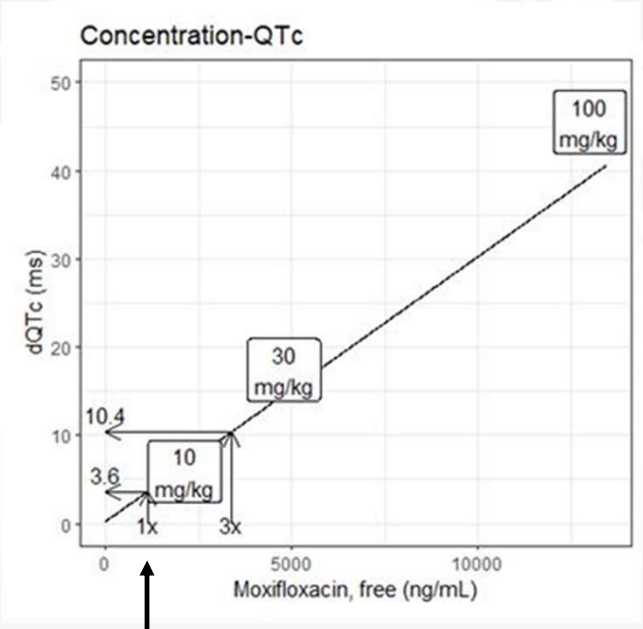
See Training Material Examples Supplemental File for additional details (Example 2)

Table 2-D: Sensitivity using historical positive control

- Minimal detectable difference (MDD)* in QT study is similar to MDD of historical positive control with same study design (XO, N=4)
  - Historical data can be used to inform exposure ratio
- Based on the C-QTc relationship for moxifloxacin, the QTc prolongation at free critical concentration (1120 ng/ml) is 3.6 ms
- Study design is ~1/3 [3.6/10] the sensitivity of clinical QT study: the study would need 3x exposure ratio to have similar sensitivity as clinical QT study

*See slide 71 for additional details on MDD and Training Material Examples Supplemental File (Example 2, Table 2-D)

MDD of historical positive control:	8 ms (95% CI: 6, 10)
MDD current in vivo QT study:	10 ms



ICH S7B Q&A 1 – Integrated Risk Assessment

- **Question 1.1: What is the general strategy for use of nonclinical information as part of an integrated risk assessment for delayed ventricular repolarization and torsade de pointes that can inform the design of clinical investigations and interpretation of their results?**

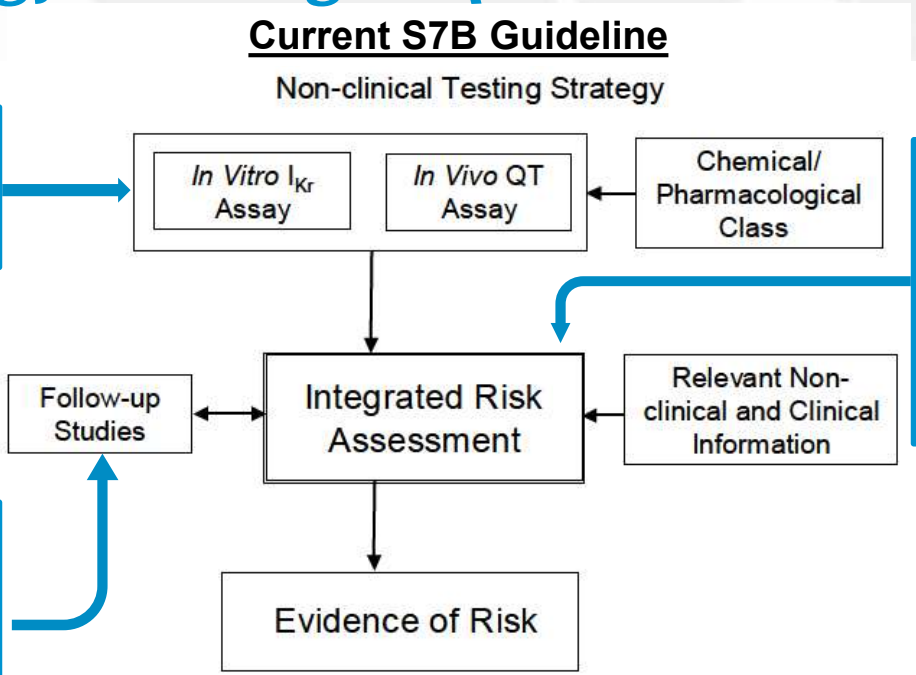
- **Question 1.2: What is the recommended method to compute the hERG safety margin?**

S7B Testing Strategy and Stage 1 Q&A Focus

“Best practice” considerations* for ion channel assays (Q&A 2.1) and ***in vivo* QT assays** (Q&As 3.1-3.5)

“Best practice” considerations for myocyte assays (Q&As 2.2-2.5)

Principles for proarrhythmia models (Q&As 4.1-4.2)



Integrated risk assessment considerations when nonclinical data is used prior to human testing vs. later in clinical development for E14 scenarios (Q&As 1.1-1.2)

**Best practice considerations are not intended to impact a sponsor’s screening activities. Some considerations only apply when using nonclinical data for clinical scenarios under E14 Q&As 5.1 and 6.1.*

Overview of the Nonclinical Integrated Risk Assessment

- **Two scenarios to use nonclinical data to inform clinical decision-making**
  - When the *in vitro* hERG assay and *in vivo* QT assay are negative
  - When the *in vitro* hERG assay and/or *in vivo* QT assay are positive
- **Principles to define hERG safety margin and *in vivo* QT best practice are provided in the Q&A**
- **Consideration should be given to the application of the 3R (replacement/reduction/refinement) principles when designing the nonclinical integrated risk assessment strategy**

Principles to Define hERG Safety Margin

- **hERG safety margin (hERG IC50 / Cmax) of a new drug is compared to the safety margin determined based on drugs known to cause TdP (more on next slide)**
  - Experimental variability should be incorporated in hERG safety margin calculations (IC50 variabilities translated to confidence interval around the safety margin)
- **hERG IC50 should be determined following Q&A 2.1 "best practice" considerations**
  - The same experimental protocol should be applied to the new drug and the reference drugs
- **Cmax = Mean steady state maximum plasma concentration when the maximum recommended therapeutic dose is given with intrinsic or extrinsic factors (high clinical exposure scenario)**
  - High clinical exposure will be an estimate early in development that is subsequently refined
  - Free (unbound) fraction of plasma concentration is usually used
    - Free fraction should be set to 1% if experimentally determined to be < 1%*
    - If protein binding cannot be accurately assessed, both free and total Cmax should be used

*consistent with regional guidance for drug interaction studies from FDA, United States; European Commission, Europe; and MHLW/PMDA, Japan

Principles to Define hERG Safety Margin

- **There are two ways for a sponsor (or a contract laboratory) to use a hERG safety margin threshold to define negative hERG assays (Q&A 2.1)**
  - **Establish** each lab's own hERG safety margin threshold using a series of reference drugs known to cause TdP (see Example 1)
    - Additional pharmacological principles or modeling (see Leishman et al. *J Pharmacol Toxicol Methods* 2020) can be used to justify a safety margin threshold
    - This theoretical justification should be supported by experimental data based on the principles in these Q&As (e.g., same experimental protocol applied to a series of drugs with known clinical TdP risk)
  - **Reuse** a safety margin threshold published by others (see Example 2, Table 1-C)
    - The published hERG safety margin should have been established following the new Q&As
    - Should demonstrate that the inter-laboratory variability of IC50s from a set of calibration drugs under the same experimental protocol does not significantly decrease the sensitivity of the published safety margin threshold (i.e., the IC50s are consistent between the laboratories)

Principles for *In Vivo* QT Assay Best Practice

- **Both the parent and any major human metabolites should be considered (ICH S7A Sec. 2.3.3.2 & 2.6)**
- **Experiments should follow general *in vivo* best practice considerations (ICH E14/S7B Q&A 3.1-3.5)**
  - e.g., species selection, heart rate correction, reporting format
- **To support E14 Q&As 5.1 and 6.1, exposures should cover the anticipated high clinical exposure scenario.**
- **To support E14 Q&A 6.1, the *in vivo* study should have sufficient sensitivity to detect a QTc prolongation effect of a magnitude similar to dedicated clinical QT studies (more on next slide)**

Principles for *In Vivo* QT Assay Best Practice

- **To demonstrate that the *in vivo* study has sufficient sensitivity to detect a QTc prolongation effect of a magnitude similar to dedicated clinical QT study in support of E14 Q&A 6.1, Q&A 3.4 lists some hypothetical examples:**
  - The minimal detectable difference might be 5 milliseconds if drug exposure in the animal study only covers the high clinical exposure
  - The minimal detectable difference might be higher if a larger multiple of high clinical exposure is achieved (e.g., 10 milliseconds if 3X high clinical exposure is achieved; or a higher QTc threshold if an even larger multiple is achieved).
- **The QTc threshold and exposure multiples selected for a particular study should be**
  - Justified by data obtained in the specific species tested
  - Using recognized reference compounds under conditions consistent with the best practice recommendations set forth in these Q&As.

Exposures Needed in Integrated Risk to Inform Clinical Decision Making

- **E14 Q&A 5.1 scenario**
  - *In vivo* QT study should cover the anticipated high clinical exposure
- **E14 Q&A 6.1 scenario**
  - *In vivo* QT study doses should cover the anticipated high clinical exposure
  - *In vivo* QT assay should have sufficient sensitivity to detect a QTc prolongation effect of a magnitude similar to dedicated clinical QT studies

Example 1: Nonclinical Data to Support E14 Q&A 5.1

- ***In Vitro* Data Summary** (refer to Table 1-B&C for E14 5.1 example for details)**:**
  - Best practice in vitro assays (Q&A 2.1) applied to 3 reference drugs with known TdP risk
  - A safety margin distribution was calculated for each reference drug taking into account the distribution of hERG IC50, the critical concentration to cause 10 ms QTc prolongation, and protein binding values
  - The upper 1-sided 95th credible interval of the safety margin distribution pooled from the 3 reference drugs defined as the safety margin threshold (51x)
  - The same assay applied to 1 concurrent positive control drug and the investigational drug
    - The positive control drug is one of the three reference drugs
    - The positive control drug IC50 is similar to the expected range under the same experimental protocol
  - The investigational drug has a safety margin of 95x; its metabolite safety margin > 3335x
    - Compare to the defined safety margin threshold of 51x from reference drugs
- ***In Vivo* Data Summary** (refer to Table 1-D for E14 5.1 example for details)**:**
  - Treatment QTc effects were consistent with vehicle effects at all dose levels (no statistical significance)
  - No QTc prolongation at 2x the high clinical exposure

Example 2: Nonclinical Data to Support E14 Q&A 6.1

- ***In Vitro* Data Summary** (refer to Table 2-B&C for E14 6.1 example for details)**:**
  - The hERG safety margin was higher (95x) than the threshold (51x) defined based on the safety margins computed under the same experimental protocol for 3 drugs known to cause TdP (same as for Example 1)
- ***In Vivo* Data Summary** (refer to Table 2-D for E14 6.1 example for details)**:**
  - Based on the historical C-QTc prolongation under the same design, the *in vivo* study has ~1/3 the sensitivity of a clinical QT study and would need an exposure margin of at least 3x to have similar sensitivity as a clinical QT study (illustrated on slide 21).
  - The current study has doses that give 5.0x the high clinical exposures.
  - Since 5.0x > 3x, the study has sufficient sensitivity to exclude QTc prolongation at high clinical exposures.
  - No QTc prolongation was observed at 5.0x the high clinical exposure scenario.

When *In Vitro* and/or *In Vivo* Assays Are Not Negative

- **Follow-up studies can be performed to further evaluate TdP risk on a case-by-case scenario**
- **Best practice considerations for some follow-up studies are described in S7B Q&As:**
  - Additional ion channels (Q&A 2.1)
  - Human derived cardiomyocytes (Q&As 2.2 - 2.4)
  - Proarrhythmia risk prediction models (can be *in vitro*, *in silico*, *in vivo*, *ex vivo*) to quantify TdP risk level (Q&As 4.1 - 4.2)
- **Follow-up studies are optional**

Follow-up Studies for Drugs Prolonging QTc Interval

- **Second Stage: S7B and E14 Q&As on how to use proarrhythmia prediction models or algorithms:**
  - To influence the design of late phase trials (e.g., intensity of ECG monitoring, eligibility criteria, stopping rules) and to inform labeling for QT prolonging drugs

- **The integrated risk assessment, including the results from follow-up studies and other relevant clinical and nonclinical information, can contribute to the design of subsequent clinical investigations and interpretation of their results**
  - First stated in the S7B guideline and re-iterated in the draft S7B Q&A 1.1

Summary

- **S7B should continue to be followed for obtaining nonclinical data to support first-in-human studies**
- **The new integrated risk assessment Q&As provide additional recommendations when nonclinical data are used later in clinical development**
  - Applying best practice (Q&As 2.1 and 3.1-3.5) is encouraged and might prevent repeat assays during clinical development
- **Double negative nonclinical assessments (*in vitro* hERG and *in vivo* QT) can be used to support E14 Q&As 5.1 & 6.1**
- **Optional follow-up studies can be used to further evaluate QT/TdP risk when nonclinical core assays are not negative**

ICH S7B Q&A 2 –
Best Practice Considerations for *In vitro* Studies

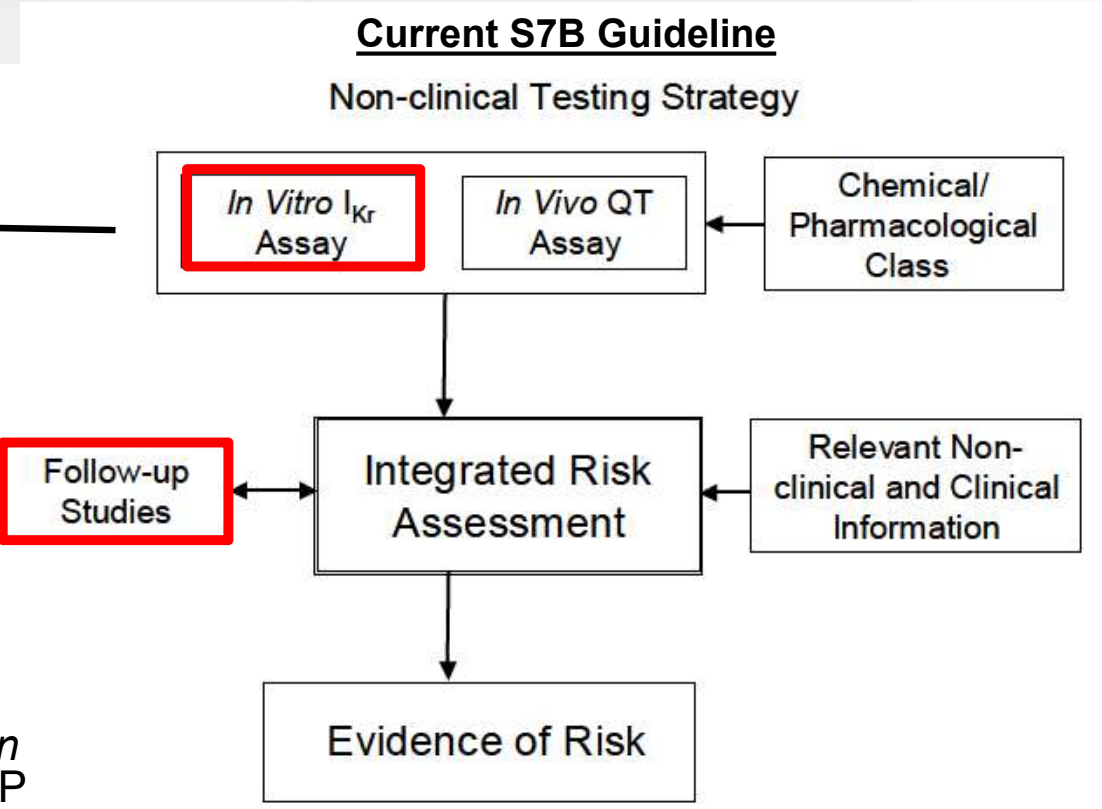
- **Question 2.1**: What are some “best practice” considerations when evaluating drug potency on affecting cardiac ionic currents using patch clamp method and overexpression cell lines?
- **Question 2.2**: What are the relevant endpoints of an informative *in vitro* human cardiomyocyte repolarization follow- up study?
- **Question 2.3**: What elements of the test system need to be considered for an *in vitro* human cardiomyocyte repolarization assay?
- **Question 2.4**: What are important considerations when designing and implementing experimental protocols for *in vitro* cardiomyocyte repolarization studies?
- **Question 2.5**: How does one define biological sensitivity of a cardiomyocyte *in vitro* repolarization assay?

Objectives of S7B Q&A #2

1. **Harmonize approaches; reduce data variability; allow for better translation to clinical findings**

2. **To incorporate conventional and newer technologies and preparations to assess drug effect on repolarization**

   An option when there is a positive signal from *in vitro* hERG/I_{kr} or *in vivo* QT assays to assist TdP risk assessment



Q&A 2.1 – Best Practices for Patch Clamp Studies

The intent of this training material is to

- To provide points-to-consider when conducting the hERG assay (and other cardiac ion channel assays) to support integrated clinical-and-nonclinical assessment under the new ICH E14/S7B Q&As
- To clarify:
  - How deviations from best practices may impact results
  - How to pick test article and positive control concentrations for testing
  - How to report data
  - Expectations of concentration recovered for analytical procedure method validation vs electrophysiology samples collected
- To provide a case study that adheres to best practices

Q&A 2.1 Recording Temperature (35 – 37˚C)

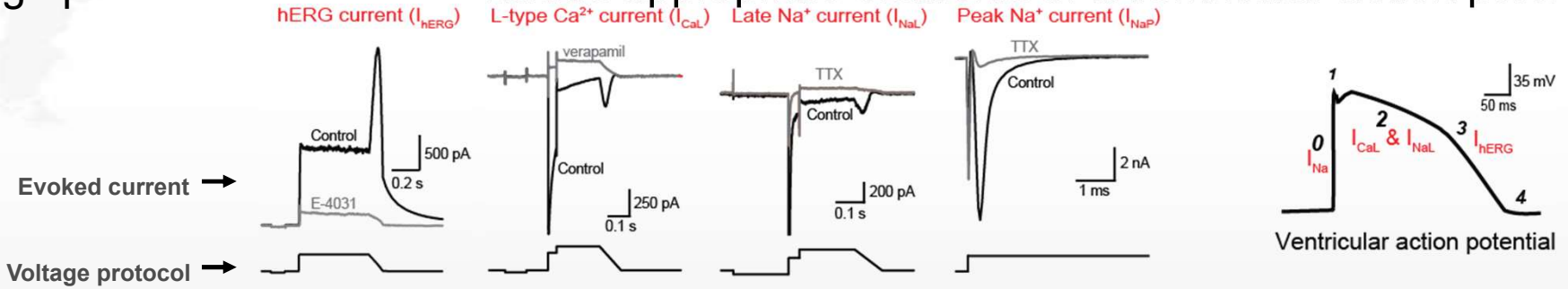
- **Points to consider**
  - The impact of recording temperature on hERG current inhibition has been reported for several molecules, including erythromycin which showed a half inhibitory concentration (IC_{50}) 7X higher at 22˚C than at 35˚C (Kirsch et al. *J Pharmacol Toxicol Methods* 2004; Guo et al. *Heart Rhythm* 2005).
  - Elevated recording temperature can lead to both increases and decreases in hERG IC_{50}s in a test article-specific manner (Alexandrou et al. *Br J Pharmacol* 2006; Kauthale et al. *J Appl Toxicol* 2015; Kirsch et al. *J Pharmacol Toxicol Methods* 2004; Yao et al. *J Pharmacol Toxicol Methods* 2005).
  - There is no method to predict physiological temperature IC_{50}s based on room temperature results.

- **Recommendation**

  Recording temperature is recommended to be at near physiological temperature (35 – 37˚C) to better predict a test article's impact on ion channels in the clinical setting.

Q&A 2.1 Voltage protocol

- **Points to consider**
  - Test article-hERG channel interactions can be state- and frequency-dependent (Stork et al. *Br J Pharmacol* 2007; Sheng et al. *Journal of Pharmacological and Toxicological Methods* 2017). Same for other cardiac ion channels (Nawrath et al. *Naunyn Schmiedebergs Arch Pharmacol* 1997; Weirich and Antoni, *J Cardiovasc Pharmacol* 1990).
- **Recommendation**
  - Voltage protocol used should include appropriate elements of a ventricular action potential

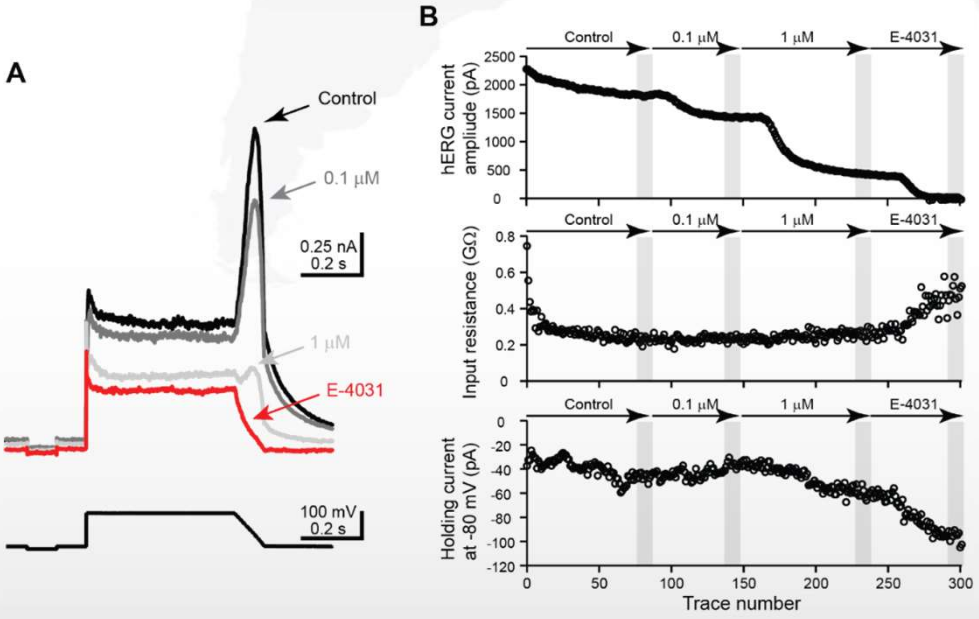


  - Use adequate pacing rate to avoid underestimating potencies of test articles that exhibit frequency-dependent block (i.e., pacing rate ≥0.2 Hz for hERG at 35-37°C).

Q&A 2.1 Recording quality

- **Points to consider:**
  - Adequate voltage control
  - Stable cell and recording properties
  - Steady state inhibition in drug solution

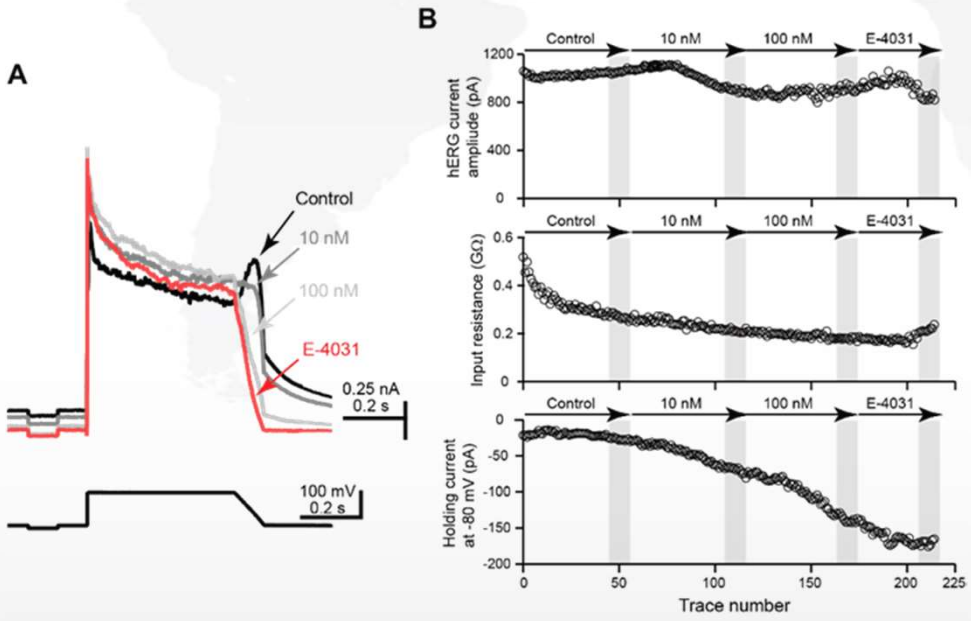
Example of adequate hERG current recording quality



A) Representative hERG traces from one cell. B) Time course plots of hERG current amplitude (E-4031-insensitive current-subtracted), input resistance, and holding current for this cell. The gray bars illustrate the data points used to estimate azimilide's effect (slide 46). Note that prior to drug application, hERG current had reached stability in control solution, and that in each azimilide concentration, drug effect also reached steady state inhibition. After baseline stability has been reached in control solution and up to the end of 1 µM azimilide application, input resistance and holding current remained stable, indicating that changes in hERG current amplitude were due to azimilide and not changes in cell properties. For this cell line, E-4031 always increased input resistance and reduced holding current. There are two possible explanations for this observation: 1) a standing hERG current, likely due to incomplete deactivation associated with this protocol, or 2) an E-4031 sensitive background current that contributes to the resting membrane potential.

Q&A 2.1 Recording quality - continued

Example of inadequate hERG current recording quality



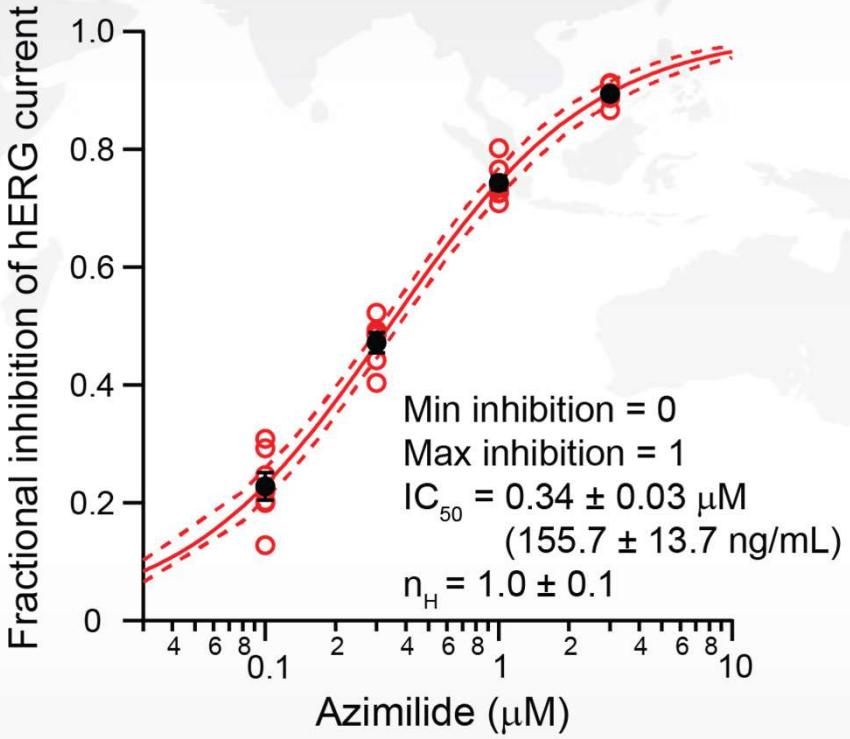
Reason – degradation of the recording quality across time.

A) This cell had very small hERG current and large background current. B) The hERG current time course plot shows inhibition by 10 nM ibutilide and no further effect by 100 nM. However, application of E-4031 at a concentration that should have eliminated hERG current still showed that much outward current due to leak current/background current remained. The continuous declines of input resistance and holding current throughout the recording suggest continuous decrease in the seal between the patch pipette and the membrane.

Note that E-4031-subtraction should not be applied to this cell to isolate the hERG current for analysis, because the background/leak current level changed sweep-by-sweep due to changes in input resistance.

Q&A2.1 Primary endpoints

- **Points to consider**
  - Half inhibitory concentration (IC_{50}), Hill coefficient (n_H), and uncertainty estimation (95% confidence interval or CI) should be reported.
  - The background current remaining after a high concentration of selective blocker application should be subtracted. Else leak current maybe calculated and subtracted from total current. If no subtraction, provide justification why this is not needed.
- **Recommendation**
  - Plot concentration-inhibition graph using individual data points and mean ± SEM (black symbols/error bars)
  - Fit individual data (red symbols) with Hill equation
  - Provide primary endpoints



Values are shown as mean ± 95% CI; dashed red curves are the upper and lower bound of 95% CI of the fit parameters. Red and black circles are defined in text on the left of this slide.

Q&A 2.1 – Test Article Concentrations

- **Points to consider**
  - S7B guideline states that “Ascending concentrations should be tested until a concentration-response curve has been characterized or physicochemical effects become concentration limiting.”
- **Recommendation**
  - Test multiple concentrations that cover the steep part of the concentration-inhibition graph (e.g., between 20 - 80% current inhibition)
  - If 50% inhibition was not achieved hence IC_{50} cannot be accurately estimated, then provide justification for not escalating concentrations (e.g. solubility limit, cellular toxicity, disruption of recording, etc).

Q&A2.1 Concentration verification

- **Points to consider**
  - Concentration of test articles exposed to cells in an *in vitro* patch clamp assay can deviate from target or nominal concentration due to molecule-specific properties, including solubility in the testing solution, stability under assay conditions, and non-specific binding to the materials used in the assay.
- **Recommendation**
  - The analytical method for the test article should be validated, as S7B states that hERG assay for regulatory submission should be GLP-compliant.
  - Solubility of test article in study buffer and stability during and after sample collection should be considered and evaluated during analytical method validation and prior to sample collection. Storage conditions and storage containers should be evaluated and specified before sample collection to avoid drug loss due to degradation or non-specific binding. Stabilization of test article immediately after collection may be required in some cases to avoid drug degradation.

Q&A2.1 Concentration verification - continued

- **Points to consider**
  - The goal of concentration verification is to understand what the recorded cell was exposed to. Do not adjust solution sample collection method to minimize deviation from nominal solution if those samples do not reflect what the cell senses during real recordings.
- **Recommendation**
  - Solution sample for analysis can be collected in ***satellite experiments***, for which the electrophysiological system is set up as if it were to be used for recordings, with temperature controller turned on, and drug solution perfused from the reservoir to the cell chamber and removed as during the real experiment. Solution samples should be collected from the cell chamber. This method is suitable ***for test articles without stability issues*** for the duration and condition of the experiments in the electrophysiology solution, and justifications should be provided for the use of satellite sample collection method.
  - *For drugs with stability issues* under the assay conditions, solution sample collection during ***real experiments*** where recordings take place is preferred to provide better estimation of exposure to the recorded cells. Solution samples should be collected at the cell chamber, as close to the site of recording or cell as possible to account for non-specific binding or degradation that can occur during real experiments.

Positive and negative controls

- **Points to consider**
  - Positive control: concentration-inhibition analysis (graphs and IC_{50} analysis) to show assay sensitivity and reproducibility across time (vs. historical data)
  - Negative control: time-matched to the longest assay to illustrate stability of operation
- **Recommendation**
  - A table summarizing historical data obtained for positive control using the same protocol and date of these experiments should be included in the study report to demonstrate data consistency across time.

Q&A2.1 Data summary

- **Points to consider**
  - Inhibition at each drug concentration for each cell should be provided in a table.
  - Provide time course plots of current amplitude, input resistance, and holding current for individual cells in control solution followed by drug solutions (see slide 39).
  - If time-dependent changes such as current run-up or run-down in baseline condition were corrected for drug inhibition estimation, the correction method should be described.

- **Recommendation**

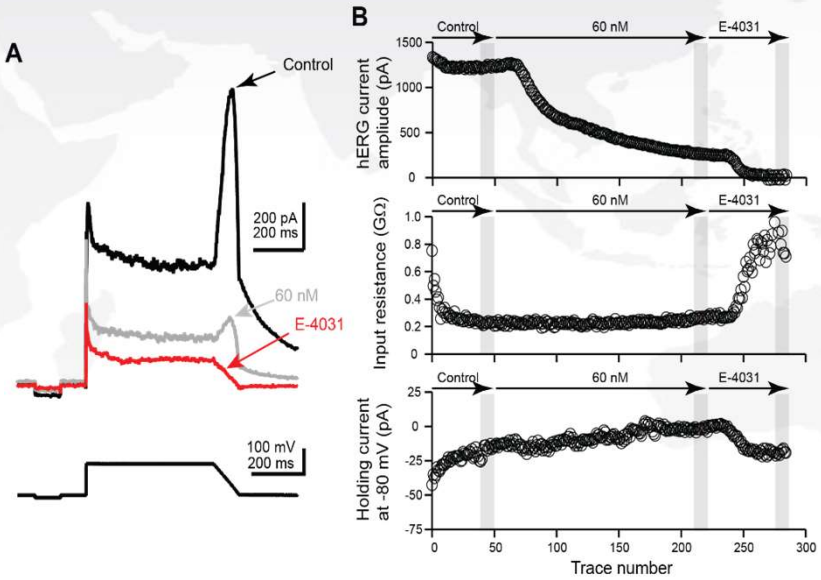
	Fractional inhibition																
Nominal [Azimilide] (μM)	Cell 1	Cell 2	Cell 3	Cell 4	Cell 5	Cell 6	Cell 7	Cell 8	Cell 9	Cell 10	Cell 11	Cell 12	Cell 13	n	Mean	SD	sem
0.1	0.25	0.13	0.20	0.20				0.31	0.29	0.22				7	0.23	0.06	0.02
0.3					0.49	0.48	0.44				0.52	0.49	0.40	6	0.47	0.04	0.02
1	0.73	0.71	0.73	0.73				0.80	0.74	0.77				7	0.74	0.03	0.01
3					0.89	0.90	0.89				0.91	0.91	0.87	6	0.89	0.02	0.01

Q&A2.1 Case study - terfenadine

Representative traces & plots to illustrate cell/recording quality.

A) Traces were from 1 cell recorded at 35-37°C and were last recorded traces in the respective solutions.

B) Time course plots for hERG current, input resistance, and holding current. It is expected that these plots are to be generated and submitted for every cell in the study report (as an appendix).



Summary table of fractional inhibition for each cell. These values were calculated using E-4031-subtracted traces.

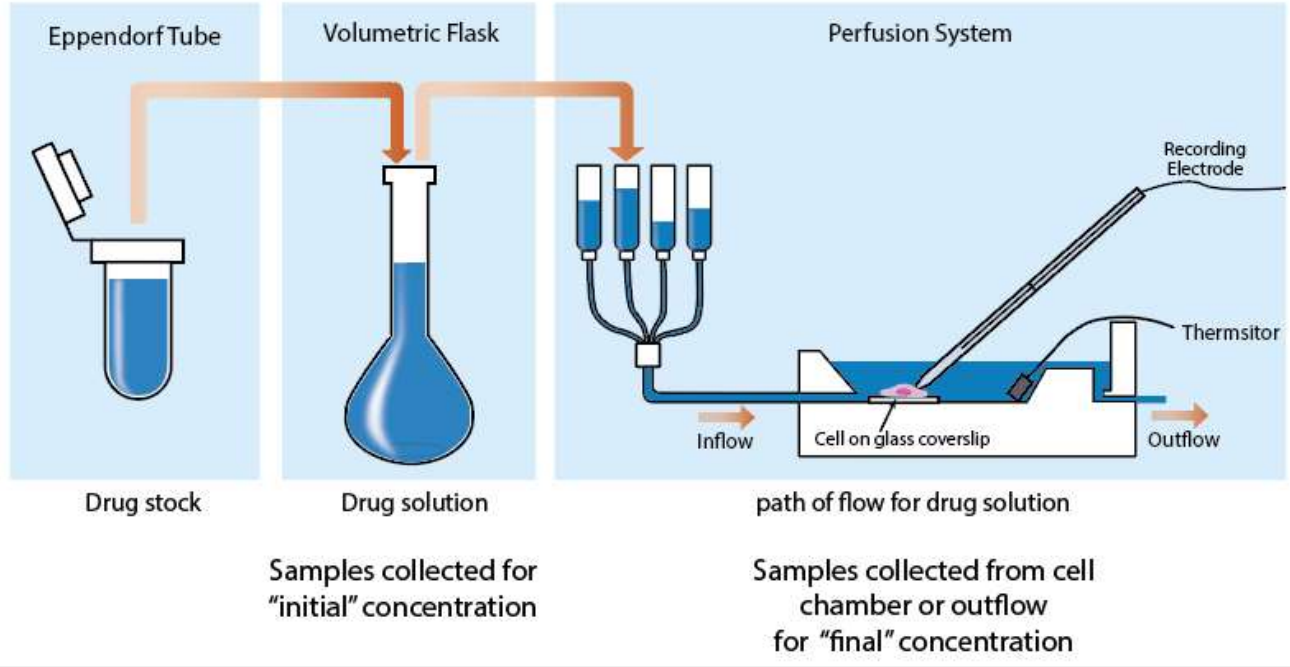
Nominal [Terfenadine] (nM)	Fractional inhibition															
	Cell 1	Cell 2	Cell 3	Cell 4	Cell 5	Cell 6	Cell 7	Cell 8	Cell 9	Cell 10	Cell 11	Cell 12	Mean	Std	Count	SEM
10			0.15	0.12	0.07	0.12	0.07	0.18					0.12	0.04	6	0.02
30		0.62								0.19	0.39	0.52	0.43	0.19	4	0.09
60	0.79								0.72		0.56	0.83	0.72	0.12	4	0.06
100			0.76	0.75	0.72	0.72		0.70					0.73	0.02	5	0.01

Q&A2.1 Case study - terfenadine

Concentration verification and reporting format:

- Electrophysiology samples were collected in satellite experiments. A validated method using LC-MS-MS was used.

- *Justification:* Terfenadine in the specific hERG electrophysiology recording solution was expected to be stable for the duration of the recordings conducted throughout the day when drug solutions were made on the day of use. Potential drug loss during recording, if any, would be due to non-specific binding.



Q&A2.1 Case study - terfenadine

Satellite sample collection method (see diagram on slide 48):

- Initial concentration refers to concentrations recovered from solution samples collected in triplicates as soon as terfenadine stock (in DMSO) was dissolved in the electrophysiology recording solution.
- Terfenadine solution was then loaded into reservoirs in the electrophysiology system and perfused normally, with temperature controlled turned on to maintain bath temperature at ~37ºC.
- Final concentration refers to concentrations recovered from solution samples collected in triplicate at the cell chamber after 5 minutes of perfusion of terfenadine solution.

Q&A2.1 Case study - terfenadine

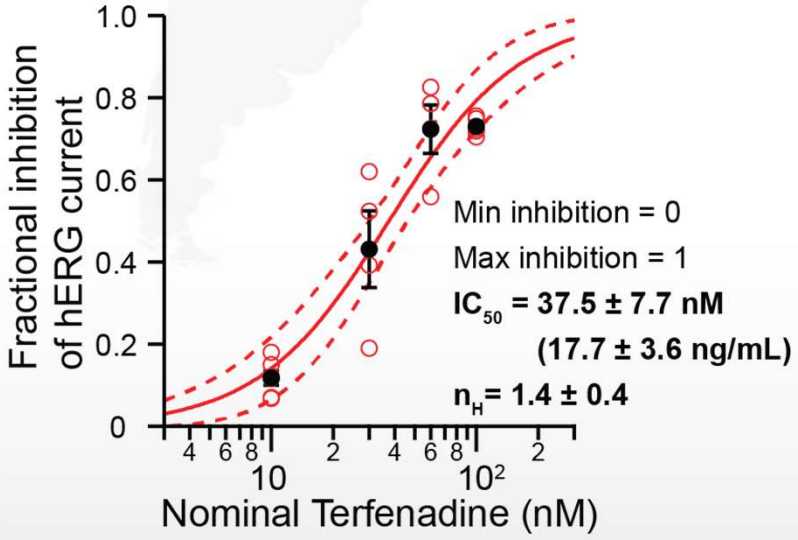
Nominal test article concentration	Initial concentration (nM)	Final concentration (nM)	% Remaining	Mean % remaining	% Loss
10 nM - Rig 1	10.15	4.84	47.7	53.5	46.5
		6.03	59.4		
10 nM - Rig 2	10.15	4.64	45.7	45.7	54.3
		4.63	45.6		
30 nM - Rig 1	26.18	12.73	48.6	52.2	47.8
		14.60	55.8		
30 nM - Rig 2	26.18	13.80	52.7	50.3	49.7
		12.51	47.8		
60 nM - Rig 1	60.38	23.75	39.3	44.7	55.3
		30.22	50.1		
60 nM - Rig 2	60.38	27.80	46.0	42.8	57.2
		23.90	39.6		
100 nM - Rig 1	95.54	38.48	40.3	39.5	60.5
		36.98	38.7		
100 nM - Rig 2	95.54	44.89	47.0	43.9	56.1
		39.01	40.8		
				Mean % loss	53.4

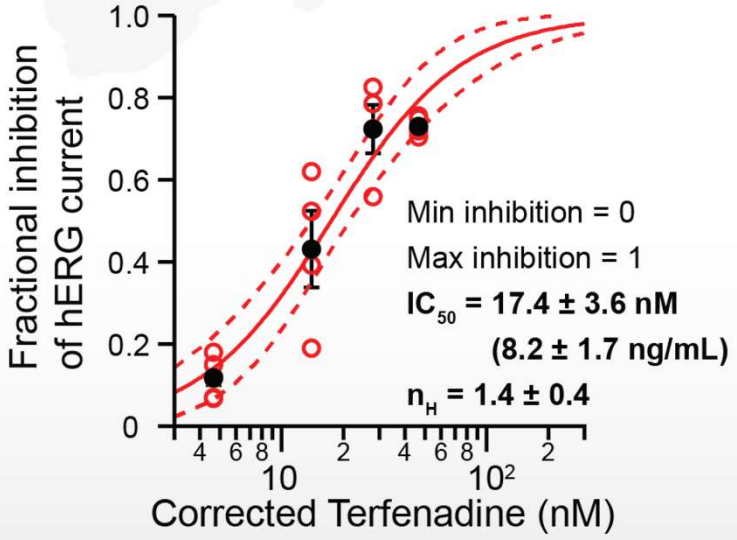
This table above summarizes analytical result for terfenadine. No concentration-dependent loss was seen. Therefore, mean % loss across all concentrations was calculated and used to adjust nominal concentrations for IC_{50} estimation.

Q&A2.1 Case study - terfenadine

Primary endpoints, uncertainty measurements, and graphical illustration.

- The graph on the left shows the plot of nominal concentration vs fractional inhibition.
- The graph on the right shows the plot of corrected concentration (after accounting for 53.4% loss) vs. fractional inhibition.



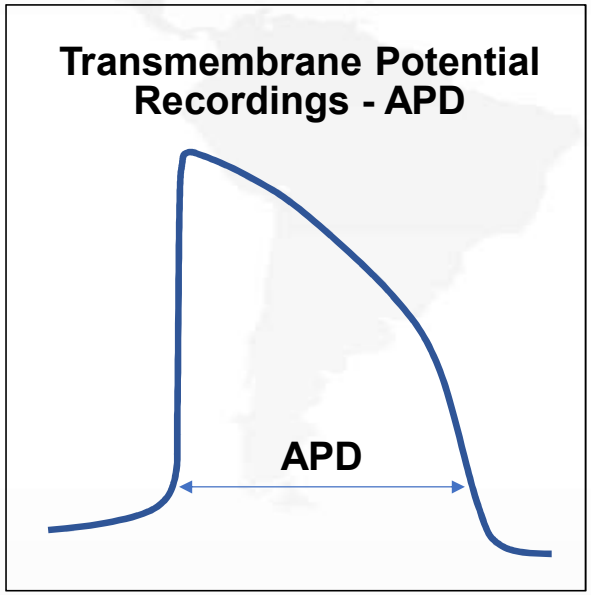


Values are shown as mean ± 95% CI; dashed red curves are the upper and lower bound of 95% CI of the fit parameters. Open red symbols are individual data points; solid black symbols and error bars are mean ± SEM.

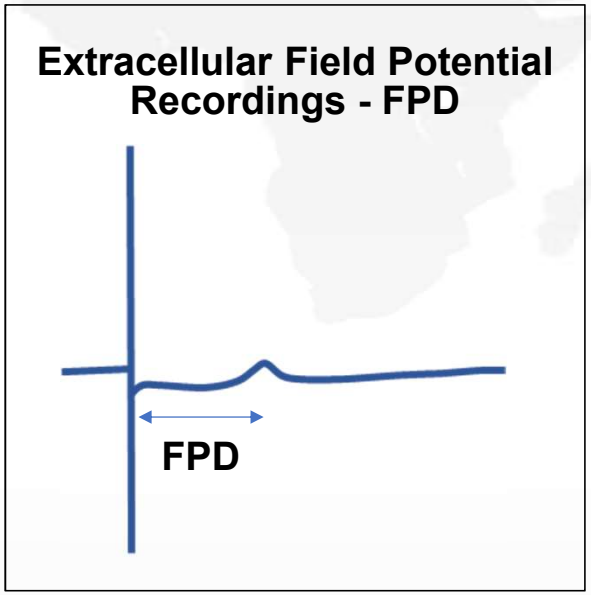
Q&A 2.1 – Overall Summary

- **S7B guideline should continue to be followed to obtain nonclinical data to support first-in-human studies**
- **The new S7B Q&A 2.1 provides best practice recommendations for patch clamp studies including the core, *in vitro* hERG assay**
  - **Harmonize approaches to enhance data reproducibility; allow for better translation to clinical findings**
  - **Provide a robust hERG safety margin for use in revised ICH E14 Q&As 5.1 and 6.1**
- **Applying best practices is encouraged; prevent repeating assays later during clinical development**

Q&A 2.2-2.5 - Electrophysiologic Study Approaches



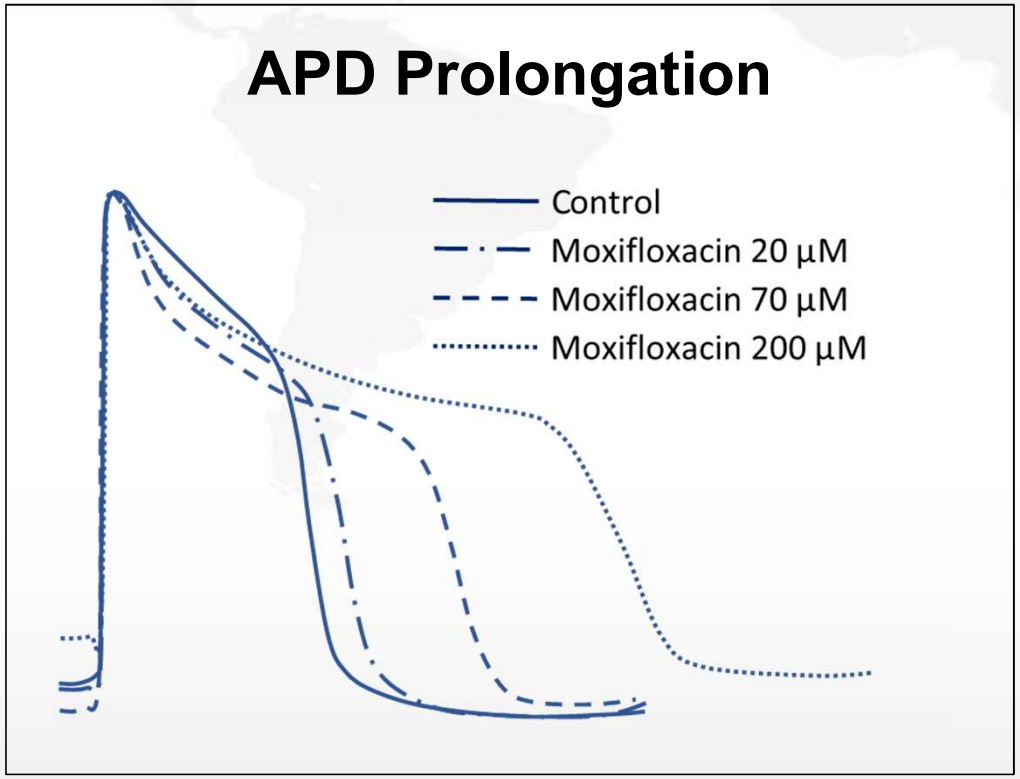
Action Potential Duration (APD)
Transmembrane potential (intracellular recordings or voltage-sensing dyes)

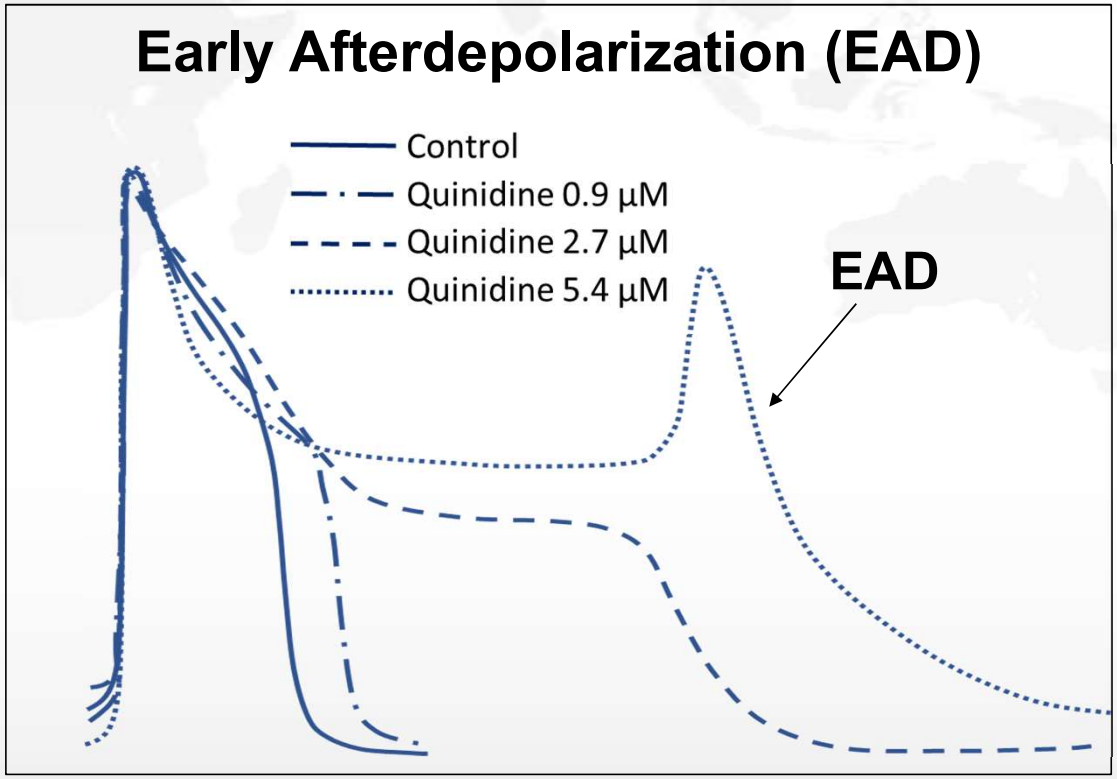


Field Potential Duration (FPD)
Multi-electrode array (MEA) recordings

- Recordings of electrical activity reflect cellular repolarization
- Calcium transients and contractility measures may provide surrogate evidence of repolarization changes downstream of electrical effects

Q&A 2.2-2.5 - Examples of Delayed or Altered Cardiac Repolarization





Adopted from Blinova et al. Tox. Sci., 155 (1), Jan. 2017, pp. 234–247 (Supplement).

Q&A 2.2-2.5 - Best Practices: Primary Repolarization Endpoints

Experimental Approach	Primary Measure	Details
Micro/Patch Electrode or Voltage Sensing Dyes	Action Potential Duration (APD)	**Primary Endpoints:** a). APD_{90} (90% of terminal repolarization) from single micro/patch electrode or optical field (voltage-sensing dyes) b). Incidence of interrupted repolarization (Early afterdepolarizations, EAD's) **Secondary Endpoint:** Repolarization intervals ($APD_{50,70,90}$) inform on time course, suggest currents affected
Multi-Electrode Array (MEA)	Field Potential Duration (FPD)	FPD typically measured from peaks of depolarization and repolarization
		Recordings from either maintaned single electrode or averaged multiple electrodes
		EAD's more difficult to detect (may appear as extrabeats); report incidence/characteristics

Vehicle, baseline, and rate-correct. Timing of post-dose sampling, sampling duration to be specified.
Justification of correction factors needed with agents affecting rhythmicity of spontaneously beating preparations.

Q&A 2.2-2.5 - Endpoints: Visual Summary Example

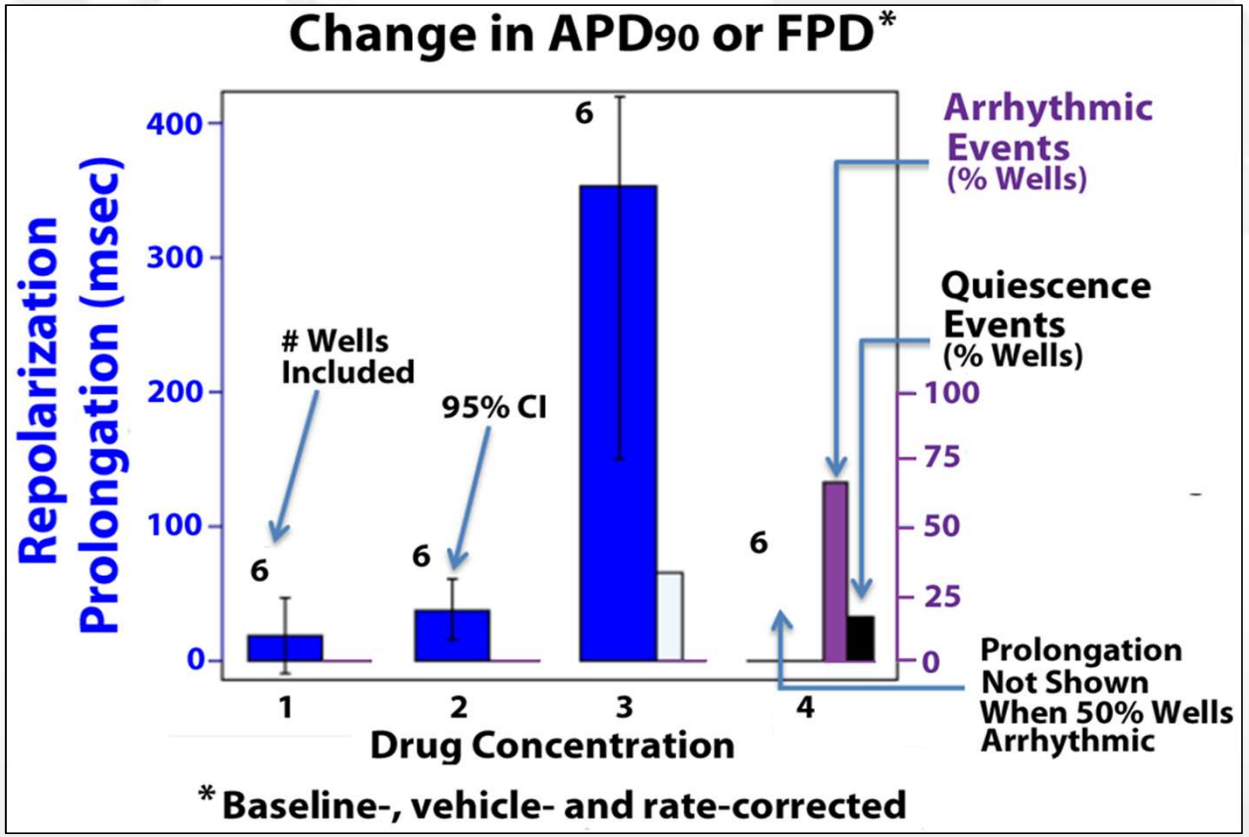


Figure modified from Blinova, et al. *Cell Reports*, 2018.

- ***Left axis***: Delayed repolarization effects with increasing concentrations
- ***Right axis***: Altered repolarization effects with increasing concentrations
- Data to be provided in tabular & graphic formats

Q&A 2.2-2.5 - Best Practices: Preparations and Experimental Conditions

- **Origins, culture conditions and baseline electrophysiological characteristics of myocytes should be well characterized and described**
  - Cell(s) sources, time in culture, substrate, media, technology platform/software
  - Criteria for acceptance of preparations/recordings (APD_{90} or FPD values [means and variability], spontaneous beat rate [for non-paced preparations])
- **Details of experimental approaches**
  - Conditions: temperature, pacing/spontaneously beating myocytes, platform
  - Stability of vehicle controls, time course of drug equilibration
  - Correction factors used (or duration of pacing), number of replicates
  - Analysis software, statistical plan
  - Validation of drug exposures in testing chambers

Q&A 2.2-2.5 - Best Practices: Characterizing Assay Sensitivity

- **Sensitivity to hERG/IKr inhibition should be demonstrated with non-saturating concentrations of selective blockers (e.g. E-4031, dofetilide)**
  - Concentration-dependent prolongation of positive control

- **Sensitivity of inhibition of depolarizing inward currents (I_{CaL} and I_{NaL}) provided when multi-channel block is suspected**
  - Repolarization shortening with I_{CaL} inhibition (e.g. nifedipine or nisoldipine)
  - Repolarization shortening with I_{NaL} inhibition (e.g. mexiletine or lidocaine)
  - Selectivity of blocking agents and potential confounding changes in spontaneous rate should be discussed

Q&A 2.2-2.5 - Overall summary: *In Vitro* Human Myocyte Repolarization Assays

- Updated best practices for *in vitro* "Follow-up Studies" (as in original ICH S7B) using human derived ventricular preparations should:
  - Guide their evolving role in comprehensive electrophysiological studies of ventricular repolarization
  - Guide information submitted to regulatory authorities for human-derived cardiomyocytes

ICH S7B Q&A 3 –
Best Practice Considerations for *In vivo* QT Studies

- **Question 3.1**: What are best practice considerations for species selection and general design of the (standard) *in vivo* QT study?
- **Question 3.2**: What should be considered for exposure assessment during the *in vivo* QT study?
- **Question 3.3**: What information is needed to support the choice of heart rate correction method in an *in vivo* QT assay?
- **Question 3.4**: How should the sensitivity of the assay be evaluated?
- **Question 3.5**: What are the recommended conventions for presenting the pharmacodynamic and pharmacokinetic results of an *in vivo* QT assay?

Introduction / Background

- Since implementation of ICH S7B, *in vivo* studies have been successful as a part of the core battery assays to safely bring investigational drugs to human studies
- A key issue is variation in the conduct, performance and QTc sensitivity of the *in vivo* QT assay, which lowers confidence in the data for clinical risk evaluation
- Over the last 15 years, lessons have been learned on how to best perform and report the results of *in vivo* QT assays, thus the "best practice" Q&As bring attention to certain considerations that add value and increase assay confidence for decision-making
- In addition, the new E14 and S7B Q&As indicate that nonclinical assays can contribute to an integrated risk assessment for TdP in later stages of development when clinical data are available. Some additional considerations apply in those scenarios.
  - Assessing drug exposure if the data will be used for E14 Q&As 5.1 or 6.1
  - Demonstrating assay sensitivity if the data will be used for E14 Q&A 6.1

Summary of *In Vivo* Best Practice Q&As

Five Q&As that cover considerations for:

- Species selection and study design (Q&A 3.1)
- Exposure assessment (Q&A 3.2)
- Heart rate correction method (Q&A 3.3)
- How should the assay sensitivity be evaluated (Q&A 3.4)
- Presenting the pharmacodynamic (PD) and pharmacokinetics (PK) results (Q&A 3.5)

- Reinforce lessons learned over the past 15 years and how methods and results should be communicated to regulators

- Highlight additional considerations for
  - Assessing drug exposure if the data will be used for E14 Q&As 5.1 or 6.1
  - Demonstrating assay sensitivity if the data will be used for E14 Q&A 6.1

Q&A 3.1: Best Practice Considerations for Species Selection and Study Design

As stated in S7B, select and justify the most appropriate non-rodent species

- **Preferable to use same species as non-rodent toxicity studies**
  - Facilitates understanding of potential relationship between cardiovascular pharmacodynamic effects and toxicity (abnormal electrolyte, pathological change, etc.)
  - Provides complementary information on exposure level (toxicokinetics)
- **Conscious freely-moving telemeterized animals are customary**
- **Alternative model (e.g., anesthetized or paced) might be justified**
  - To achieve adequate exposure
  - To overcome drug-related challenges (e.g., heart rate change, tolerability, bioavailability limitation)
  - Species selection and general *in vivo* study design should be in accordance with the 3R (replacement/reduction/refinement) principles

Q&A 3.2: Considerations for Achieving Adequate Drug Exposure

- **S7B states that drug exposures should include and exceed anticipated therapeutic concentrations**
- **If the data are to be used to support clinical decision making under ICH E14 Q&As 5.1 or 6.1, the exposure should cover the anticipated high clinical exposure scenario**
  - **Defined (see E14 Q&A 5.1) as exposure in patients (Cmax, steady state) when the maximum therapeutic dose is given with intrinsic (e.g., renal/hepatic impairment) or extrinsic (e.g., drug-drug interactions) factors**
  - **As noted in ICH S7B, the dose range can be limited by animal intolerance to the test substance**

Q&A 3.2: Considerations for Assessing Drug Exposure

- **Assessing exposure in the same animals used for QT assessment is encouraged, but can be done in separate animals**
  - **Exposure data from a separate PK and toxicity study can be used**
- **Blood samples should be taken at relevant time-points and in a manner that limits interference with QT assessment**
  - **Can be done by sampling complete PK profiles in the same animals on a separate day after an adequate washout**
  - **By using limited (e.g., 1-2) samples from the QT assessment day to demonstrate consistency with full pharmacokinetic profiles generated in different animals in a separate study**

Q&A 3.2: Considerations for When to Utilize Exposure-Response Modeling

- **If sufficient PK sampling is performed, exposure-response modeling similar to concentration-QTc analysis for clinical QT studies can be performed**

- **This can be helpful when the nonclinical *in vivo* QT assay should be powered to detect an effect similar to dedicated QT studies in humans as it can reduce the number of animals in accordance with the 3R (reduce/refine/replace) principles**
  e.g., when using *in vivo* QT data to support clinical decision making under ICH E14 Q&A 6.1

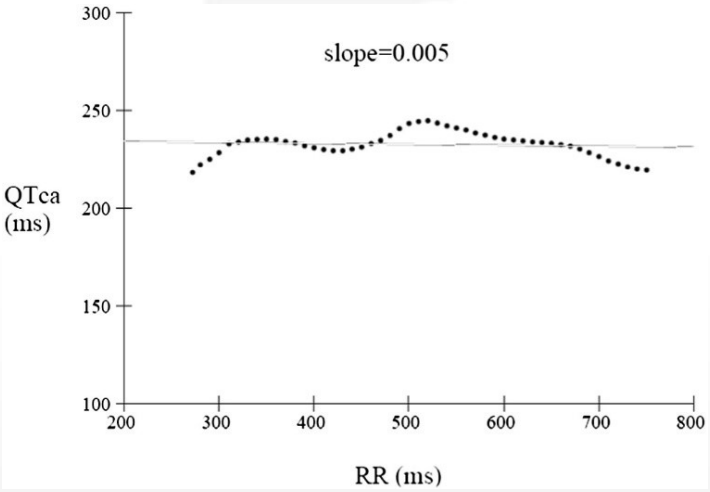
- **In addition, exposure-response modeling may be helpful in other circumstances when QT prolongation is observed or anticipated based on hERG assay results**

- **Representative references for nonclinical *in vivo* concentration-QTc modeling**
  - **Dubois et al. *British Journal of Clinical Pharmacology* 2017, Komatsu et al. *Journal of Pharmacological and Toxicological Methods* 2019, Chui et al. *Clinical and Translational Science* 2021**

Q&A 3.3: Best Practice for Heart Rate (HR) Correction Method

Independence of QTc to RR intervals should be demonstrated through QTc versus RR plots accompanied by additional information

Example plot demonstrating independence of QTc vs. RR



QTca: Individual rate-corrected QT (see next slide)

Figure reproduced from Holzgrefe et al. *Journal of Pharmacological and Toxicological Methods*, 2014 with permission from Elsevier.

Additional information

- **Number of matched QTc-RR pairs**
- **Correlation metric**
- **95% confidence interval**
- **P-values**

Q&A 3.3: Individual QT Correction Based on QT-RR Relationship is Preferred with Drugs that Affect Heart Rate

Individual rate-corrected QT (QTca) is best practice and recommended when there are a sufficient number of QT-RR pairs and a broad range of RR values (obtained from vehicle-treated animals)

Example of individual QT correction

- **$QTca = RR_{ref}^{\beta} \times QT_{raw} / Rr_{raw}^{\beta}$** (Miyazaki H & Tagawa M, 2002)
- **$QTca = QT_{raw} / (QT_{raw} / RR_{ref})^{\beta}$** (Holzgrefe H. *et al.*, 2014)

Conventional HR correction methods should be avoided or validated if used

- **e.g, QTcV (Van de Water), QTcF (Fridericia), QTcB (Bazett)**

Q&A 3.4: Assessing Assay Sensitivity – General Recommendations

- **The test system should provide a robust response**
- **Assay sensitivity of relevant functional endpoints should be evaluated and reported to enable data interpretation and contextualization**
  - **Demonstration of assay sensitivity can be achieved by defining minimum detectable differences and testing the effects of positive controls**
  - **Statistical power calculations could also be provided from historical data from the same laboratory using the identical protocol**
    - If historical positive control data are utilized to justify assay sensitivity or statistical power is calculated from historical control data, then the variance of the present data should be consistent with that seen historically

Minimum Detectable Difference (MDD)

- **The minimal detectable difference (MDD) is a statistical indication of the smallest effect size that can be determined in a QTc assay**
  - A retrospective power analysis is used to determine MDD for a given study
  - A historical evaluation of study-specific MDD values can be used to track the sensitivity and reproducibility of QTc signal detection over many studies

- ***Example*:** Baublits et al. perform a retrospective statistical power analysis of multiple studies across different species and demonstrate that small QTc effects (< 10 ms) could be detected
  - See Baublits et al. *Journal of Pharmacological and Toxicological Methods*, 2021 for addtional details

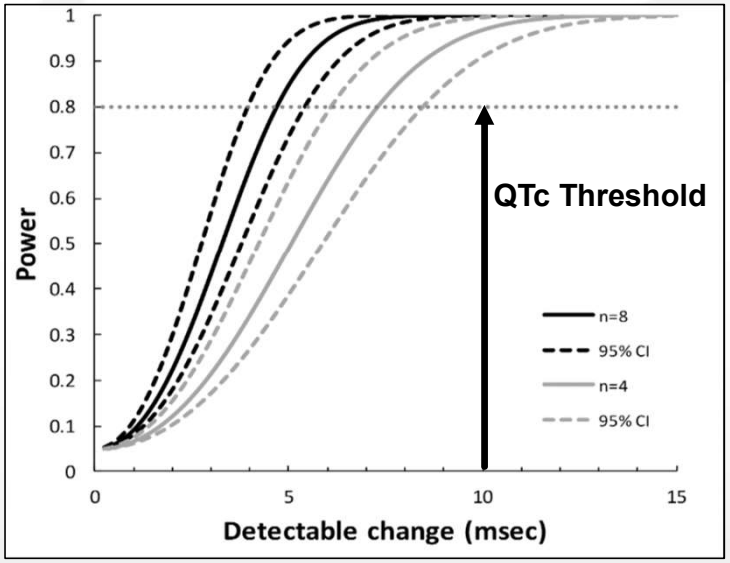


Figure reproduced from Baublits et al. *Journal of Pharmacological and Toxicological Methods*, 2021 with permission from Elsevier.

Q&A 3.4: Assessing Assay Sensitivity – Additional Considerations

- **Currently, as a positive control is not routinely used in the *in vivo* QT assay, assay sensitivity is commonly validated when introducing or changing the test system (e.g., ECG system, species) in each laboratory**

- **If study results are to be used to support an integrated nonclinical and clinical risk assessment described in ICH E14 Q&A 6.1, then the study should have sensitivity to detect a QTc prolongation effect of a magnitude similar to dedicated clinical QT studies, taking into consideration inter-species differences in the normal range of values for the QTc interval**

Q&A 3.4: Considerations When Assessing Assay Sensitivity in Support of ICH E14 Q&A 6.1 Scenarios

- **The overall sensitivity of the nonclinical assay in comparison to clinical QT studies depends on both the electrocardiographic assessment and the exposure achieved in the *in vivo* assay relative to high clinical exposure**
- **Hypothetical example presented in the Q&A:**
  - The minimal detectable difference (MDD) might be 5 milliseconds if drug exposure in the animal study only covers the high clinical exposure
  - A higher MDD might be considered adequate if a larger multiple of high clinical exposure is achieved
    - e.g., 10 milliseconds if 3X high clinical exposure is achieved
    - or a higher QTc threshold if an even larger multiple is achieved
- **Higher exposures can help reduce the numbers of animals used in accordance with the 3R (reduce/refine/replace) principles**

Q&A 3.4: Use of a Positive Control to Demonstrate Sensitivity

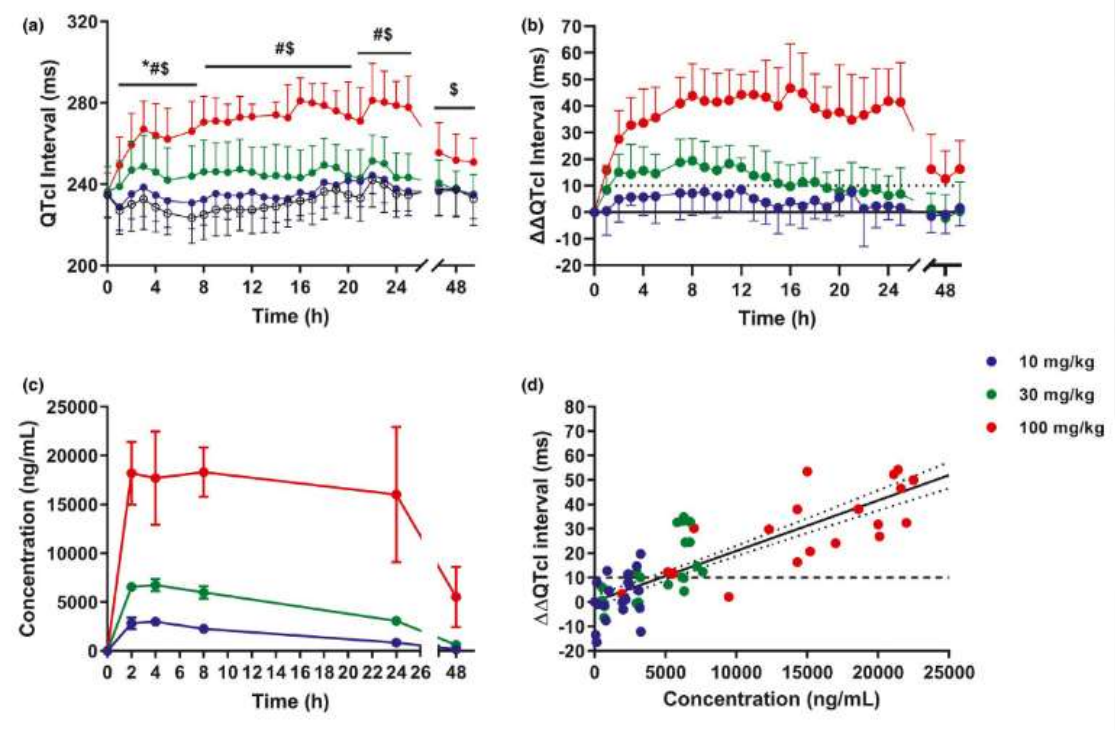


Figure 1 Time-response and concentration-QTc (C-QTc) relationship evaluation of moxifloxacin-induced QTc prolongation in conscious beagle dogs. Vehicle (○) and moxifloxacin (10, 30, and 100 mg/kg) were administered at 0 h. The plots represent timepoint analysis of absolute QTcI (a) and baseline- and vehicle-corrected QTcI effects (ΔΔQTcI) (b) following treatment. The moxifloxacin pharmacokinetic curve (c) and C-QTc relationship for moxifloxacin (d) are also shown. Group sizes were eight (a/b) or four (c/d) and values are mean ± SD. *Indicates significance ($p < 0.05$) for control versus low dose. The # indicates significance ($p < 0.05$) for control versus mid dose. The $ indicates significance ($p < 0.05$) for control versus high dose (repeated measures analysis of covariance followed by Dunnett's pairwise comparisons). For panel d, data were fitted by linear regression (solid line) and dotted lines represent 90% confidence interval of the model-predicated mean ΔΔQTcI.

***Example*: Moxifloxacin was tested to demonstrate QTc sensitivity with by time-response and concentration-QTc (c-QTc) analysis:**

- Time-response analysis (Fig. 1a-c): dose-related prolongation of QTcI intervals observed at clinically-relevant exposures; PK analysis was conducted in telemetry study animals. The low dose (10 mg/kg) of moxifloxacin increased QTcI intervals by 5.9 ms ($p<0.05$) at Cmax of 2980 ng/ml (total).

- C-QTc analysis (Fig. 1d): linear-regression demonstrated clinically-relevant detection sensitivity. A 10 ms change was estimated at a total plasma concentration of 4627 ng/ml.

- Conclusion: Free concentrations of moxifloxacin that produce a 10 ms QTc change were 2 to 2.5-fold larger than human thorough QT study data.

Figure reproduced from Chui et al., *Clinical and Translational Science* 2021 with permission from Elsevier

Q&A 3.5: How to Present PD and PK Results of *In Vivo* QT Assay

PD content

Summary table and figures showing

- Absolute mean value, mean absolute and percent change from baseline, confidence interval
- P-value for changes from baseline and vehicle control

If study results are being used to support ICH E14 Q&A 6.1

- Report minimal detectable differences with by time analysis
- providing that the data for the new drug and the historical data were collected according to the same protocol and statistical analysis plan

If concentration-QTc modeling is performed;

- **Reporting should follow similar principles as for human concentration-QTc modeling**

PK content

Summary statistics for Cmax, AUC and Tmax for parent drug and metabolite (by table)

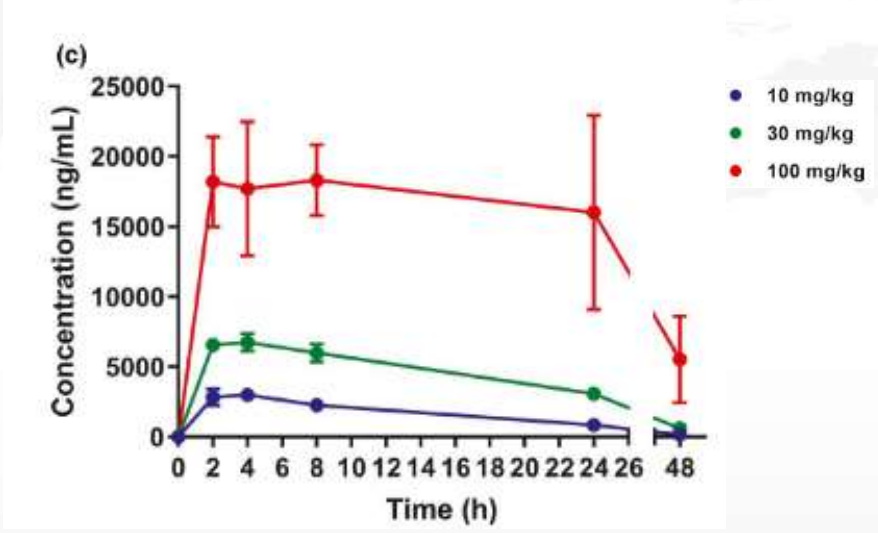
Time plot vs. plasma concentration for parent drug and metabolite (by figure)

Q&A 3.5 PK Data Summary for *In Vivo* QT Assay: Illustrative Example

The PK data may include individual animal data, summary statistics (e.g., C_{max}, AUC, T_{max}) and plasma concentration time-plots for the parent drug and metabolite.

- ***Example*: Plasma concentrations and time-plot of moxifloxacin**

Dose (mg/kg	Hours	Animal 1	Animal 2	Animal 3	Animal 4	Total plasma Mean (SD) (ng/ml)	AUC (0-48 h) Mean (SD) (ng*h/ml)
10	0 2 4 8 24 48	11.7 3110 3090 2470 1010 242	3.5 1960 2380 2200 798 84	9.4 3270 3190 2010 684 154	9.0 2980 3250 2370 897 167	8.4 (3.5) 2830 (590) 2980 (405) 2260 (202) 847 (139) 162 (65)	52000 (6440)
30	0 2 4 8 24 48	4.8 6410 6740 6170 2960 533	25.0 7230 7630 5190 3110 675	15.0 6290 6230 5830 3240 700	26.5 6300 6320 6720 2950 527	17.8 (10.1) 6560 (449) 6730 (640) 5980 (643) 3070 (138) 609 (91)	151000 (1710)
100	0 2 4 8 24 48	10.2 20000 22000 18600 22500 5550	6.8 21600 21400 18600 14300 5180	12.6 17000 12300 15000 7010 1930	11.9 14300 15200 21100 20100 9470	10.4 (2.6) 18200 (3210) 17700 (4780) 18300 (2520) 16000 (6920) 5540 (3090)	633000 (194000)



Moxifloxacin plasma concentration.
Symbols (ng/ml) are mean ± SD.

Data sources: Chui R et al., 2021 and Amgen Study No 114803
(Figure reprinted with permission from Elsevier)

Other: How to define QT positive and negative

- **Statistical analysis of QTc by time**
  - Assay sensitivity is highly dependent on the experimental design and statistical methodology utilized
  - A sensitive statistical methodology, such as analysis of variance (ANOVA), is recommended for study designs that assess treatment, animal and period effects
    - ANOVA can be applied to both cross-over designs and parallel group designs
    - Representative references: Aylott et al. (2011), Derakhchan K et al. (2014), Chui R et al., (2021)

- **Examples of Statistical Analysis:**
  - **Positive Effect: statistical significance for drug treatment effect**
    - Drug treatment produces QTc effects that are dose-dependent or time-dependent
    - Representative example: Moxifloxacin profile in dogs (see slide 74)

  - **Negative Effect: no statistical significance following drug treatment**
    - Drug treatment effects are consistent with vehicle-treatment; no QTc effect observed
    - Representative example: Levocetirizine profile in monkeys (see Komatsu et al. 2019)

3Rs Principles

- **Consideration should be given to design features, ECG methodologies, and statistical approaches that can reduce the sample size needed to achieve the desired sensitivity targets**
  - Ideally, a single well-designed assay would support first-in-human studies (S7A/B) *and* enable an integrated QTc risk assessment for scenarios in E14 Q&A 6.1, if the latter is to be pursued
- **References to example studies in the literature are provided only to illustrate factors that impact and improve performance of the *in vivo* QT assay and not to recommend specific design elements**
  - The Sponsor should use "fit to purpose" study designs to achieve specific study goals
- **Sponsors should include all relevant data that support *in vivo* QT assay best practices in regulatory submissions (e.g., study reports)**
  - Justification for group size selection, in accordance with the 3Rs, should be provided

Summary

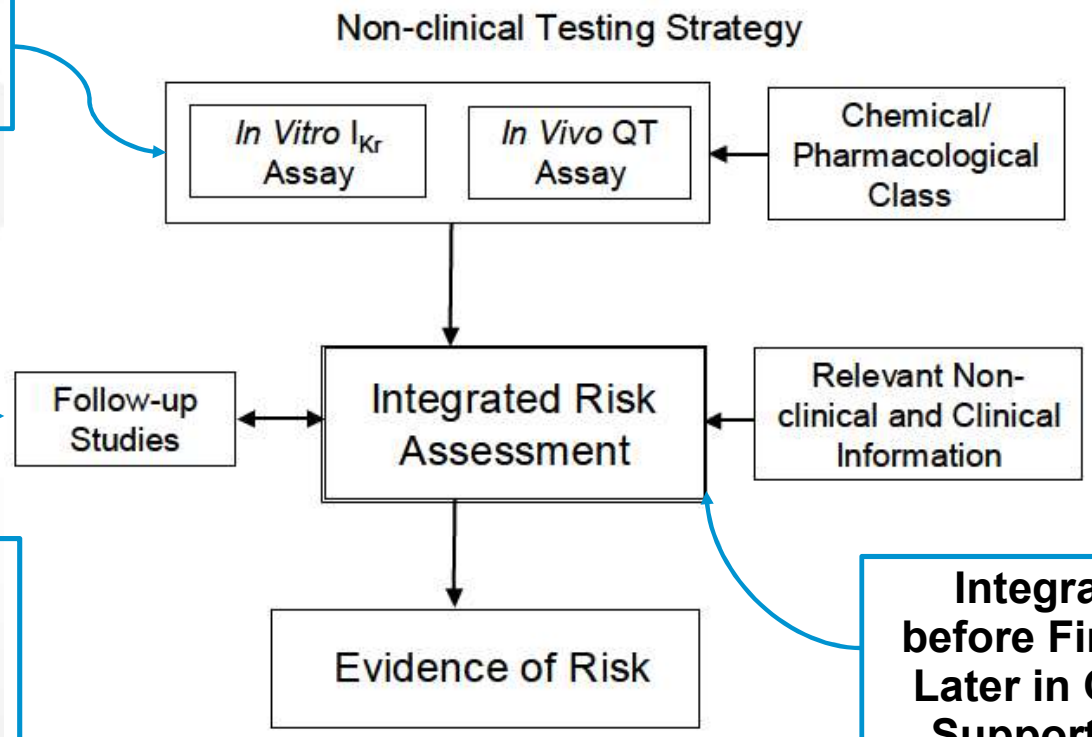
- **Q&A 3.1, species selection and study design**
  - Conscious freely-moving telemeterized non-rodent animals are customary
- **Q&A 3.2, exposure assessment**
  - Exposure-response modeling may be helpful in certain circumstances
- **Q&A 3.3, heart rate correction method**
  - Individual rate-corrected QT (QTca) is suitable, when a drug affects HR.
- **Q&A 3.4, assay sensitivity**
  - Recommendations for assessing assay sensitivity, including defining MDD and testing the effects of positive controls
- **Q&A 3.5, presenting the PD, PK and demonstration of assay sensitivity results**

ICH S7B Q&A 4 – Principles for Proarrhythmia Models

- **Question 4.1**: The ICH S7B guideline (Section 3.1.4) states that directly assessing the proarrhythmic risk of pharmaceuticals that prolong the QT interval would be a logical undertaking and interested parties are encouraged to develop these models and test their usefulness in predicting risk in humans. What are general principles to evaluate whether a proarrhythmic risk prediction model could be used as part of an integrated risk assessment strategy?
- **Question 4.2**: How can a sponsor use a model for regulatory submission and what are the limitations?

Background

"Best Practice" hERG/Ikr & *in vivo* QT Assays



"Best Practice" Myocyte Assays & Principles for Proarrhythmia Models

Integrated Risk Assessment before First-in-Human Dosing vs. Later in Clinical Development to Support Clinical Interpretation

Definition of Proarrhythmia Model

- **Models using nonclinical experimental data as input and generating human proarrhythmia risk prediction as output**
- **Examples for model input:**
  - *In silico* – ion channel data
  - *In vitro* – ion channel data, drug-induced repolarization change/arrhythmia
  - *Ex vivo/in vivo* – drug induced action potential or ECG parameter change/arrhythmia
- **Examples for model output:**
  - Proarrhythmia metrics

Examples of Model Input and Output

Model	Input	Output	Ref.
In silico Single ventricular model	Ion channel data	Net current charge (qNet)	Li et al., *Clinical Pharmacology and Therapeutics,* 2018
In vitro iPSC-cardiomyocyte	Field potential change, Ratio between proarrhythmia and therapeutic concentration	2-dimentional map with risk categories	Ando et al., *Journal of Pharmacological and Toxicological Methods,* 2017
Ex vivo Purkinje fiber	Action potential parameter changes, reverse-use dependency, others	Proarrhythmia score	Champeroux et al., *British Journal of Pharmacology,* 2005

Principles for proarrhythmia models used for regulatory purpose

- **The following general principles should be applied to all proarrhythmia risk prediction models intended to be used as part of an integrated risk assessment for regulatory purposes:**
  - Defined endpoint
  - Fully disclosed algorithm
  - Defined domain of applicability/ scope and limitations
  - Prespecified analysis plan and criteria
  - Mechanistic interpretation
  - Uncertainty
- **Above six principles follow the white paper (Li. et al. *Clinical Pharmacology & Therapeutics*, 2019)**

Principles in S7B Q&A

- **Defined endpoint**
  - For TdP risk prediction, a series of reference drugs with known clinical TdP risk should be used to define the endpoint
- **Fully disclosed algorithm**
  - Enough transparency for independent evaluation of the model
- **Defined domain of applicability/scope, context of use and limitations**
  - Defined/standardized experimental protocol
  - Understand what mechanisms are covered (and not covered) by the model

Principles in S7B Q&A

- **Prespecified analysis plan and criteria**
  - Training and validation steps should be separated
  - Before validation, performance metric and acceptable criteria should be specified
- **Mechanistic interpretation**
  - Need to describe the relationship between model inputs (experimental measurements) and mechanism of arrhythmia
- **Uncertainty quantification**
  - The uncertainty in the model inputs (experimental measurements) should be captured and propagated to the model predictions

Model qualification and implementation

- After a model has been developed, a process should be followed to evaluate whether the model development complied with 6 principles
- Model developers are encouraged to contact a regulatory agency for formal qualification
- After a model has been qualified, the model can be used by other labs within the same context of use
- New labs should use a subset of the reference compounds to internally calibrate and validate the model
- Examples of selecting the subset calibration compounds, performing lab-specific calibration and validation: Han et al. *Journal of Pharmacological and Toxicological Methods* 2020

Model implementation process

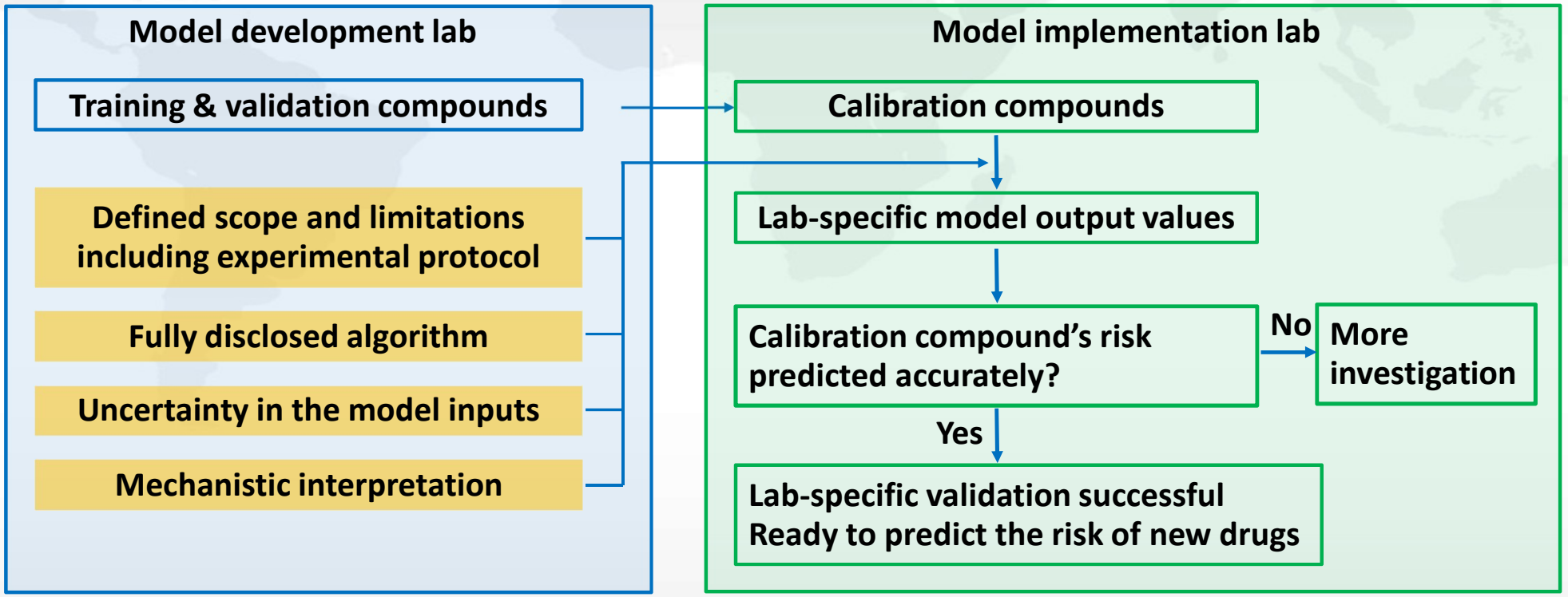


Image modified from Han et al. *Journal of Pharmacological & Toxicological Methods*, 2020

Example: The Use of Follow-up Studies

As described in S7B Q&A 1.1:

- If the hERG assay and/or the *in vivo* QT study suggest an effect at clinical exposures, the drug has a risk of interfering with ventricular repolarization
- Follow-up studies could be performed to further explore the mechanisms and assess the TdP risk
- The assessment of TdP risk using follow-up studies, although optional, can be used together with other relevant nonclinical and clinical information to contribute to the design of subsequent clinical investigations and interpretation of their results

Summary

- A proarrhythmia risk assessment model could be used as follow-up investigation when nonclinical core assays are not negative (part of the integrated risk assessment)
- The model should have been qualified under consideration of the general principles presented in the Q&A
- The models can be used not only by developer(s) but also by other labs with lab-specific validation data

OVERALL SUMMARY

Summary of Q&A Document Content

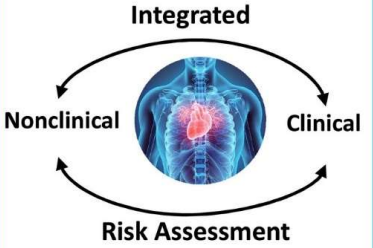
- **Revised E14 Q&As that incorporate how to use an integrated nonclinical-clinical assessment to:**
  - Reduce the number of clinical trials by allowing for more 'TQT substitutes' (E14 Q&A 5.1)
  - Reach a low-risk determination when a TQT or equivalent cannot be performed (E14 Q&A 6.1)

- **S7B Q&As that expand on the nonclinical components of the integrated nonclinical-clinical assessment to support the E14 Q&A 5.1 and 6.1 scenarios and on the use of follow-up studies when indicated**

Integrated Risk Assessment (S7B Q&As 1.1-1.2)

Double-negative hERG and *in vivo* QT assays to:

- Reduce the number of clinical trials by allowing for more 'TQT substitutes' (**E14 Q&A 5.1**)
- Reach a low-risk determination when a TQT or equivalent can't be performed (**E14 Q&A 6.1**)



Integrated assessment of core assays and follow-up studies for non-double negative scenario (S7B Q&A 1.1):

- Contributes to design of clinical studies and interpretation of their results (e.g., mechanism of clinical QTc prolongation)
- Case-by-case evaluation for clinical/regulatory decision making (e.g., E14 Q&A 7.1)

Figure reproduced from Strauss et al. *Clinical Pharmacology & Therapeutics*, 2021

Summary of Q&A Document Content

- **Best practice considerations for nonclinical assays**
  - **Core assays:**
    - *In vitro* hERG assay
    - *In vivo* QT assay
  - **Follow-up studies, including with new technologies:**
    - *In vitro* cardiomyocytes (including induced pluripotent stem cell-based assays)
    - Proarrhythmia models (including laboratory or *in silico* proarrhythmia models)

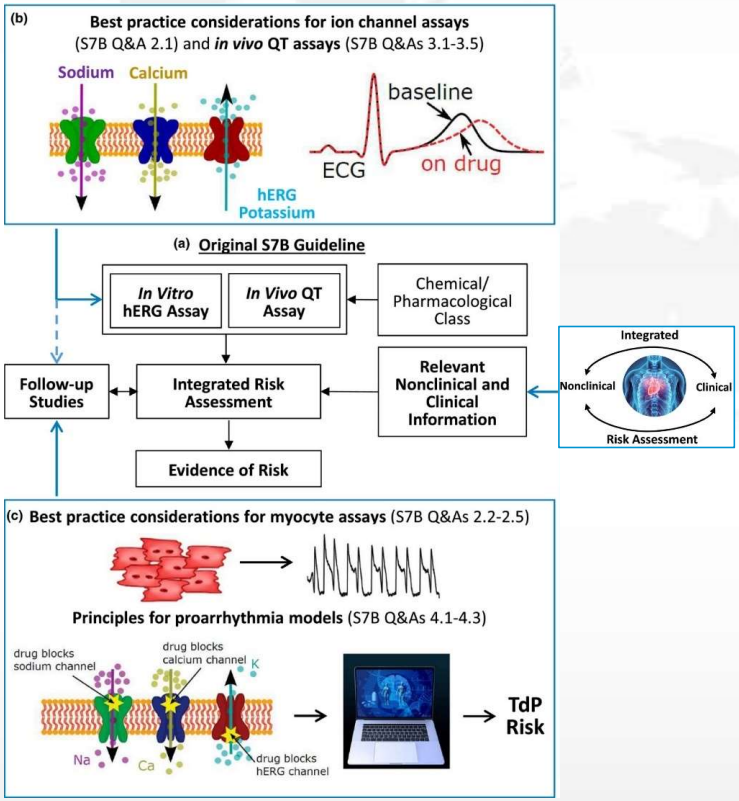


Figure reproduced from Strauss, et al. *Clinical Pharmacology & Therapeutics*, 2021

Conclusions

- While at adoption E14 suggested a QT interval evaluation independent of S7B results, both documents highlighted the need for integration of information in a manner which is informative as a totality of evidence
- These new E14 and S7B Q&As are directed at scenarios where nonclinical data can reduce the number of clinical studies and inform clinical regulatory decision making at the time of a marketing application
- These Q&As also outline best practices and principles for new and existing *in vitro* and *in silico* models that have the potential to enhance the prediction of the risk for human proarrhythmia risk
- Consideration is given to the 3R (reduce/refine/replace) principles

Contact

- **For any questions please contact the ICH Secretariat:**

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