

# **BEST PRACTICE CONSIDERATION FOR THE *IN VITRO* CARDIAC ION CHANNEL AND *IN VIVO* QT STUDIES (S7B Q&A, BEST PRACTICE)**

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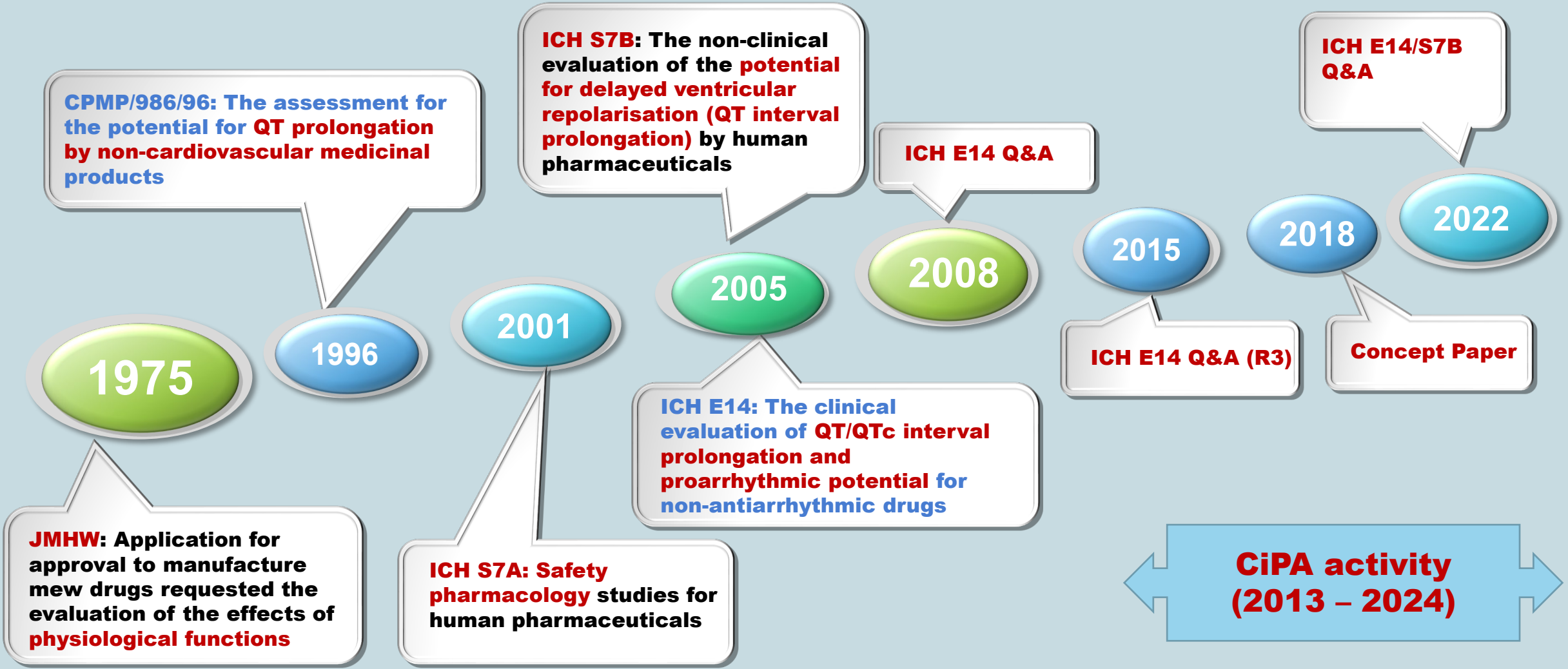
Korea Institute of Toxicology



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# CONTENT

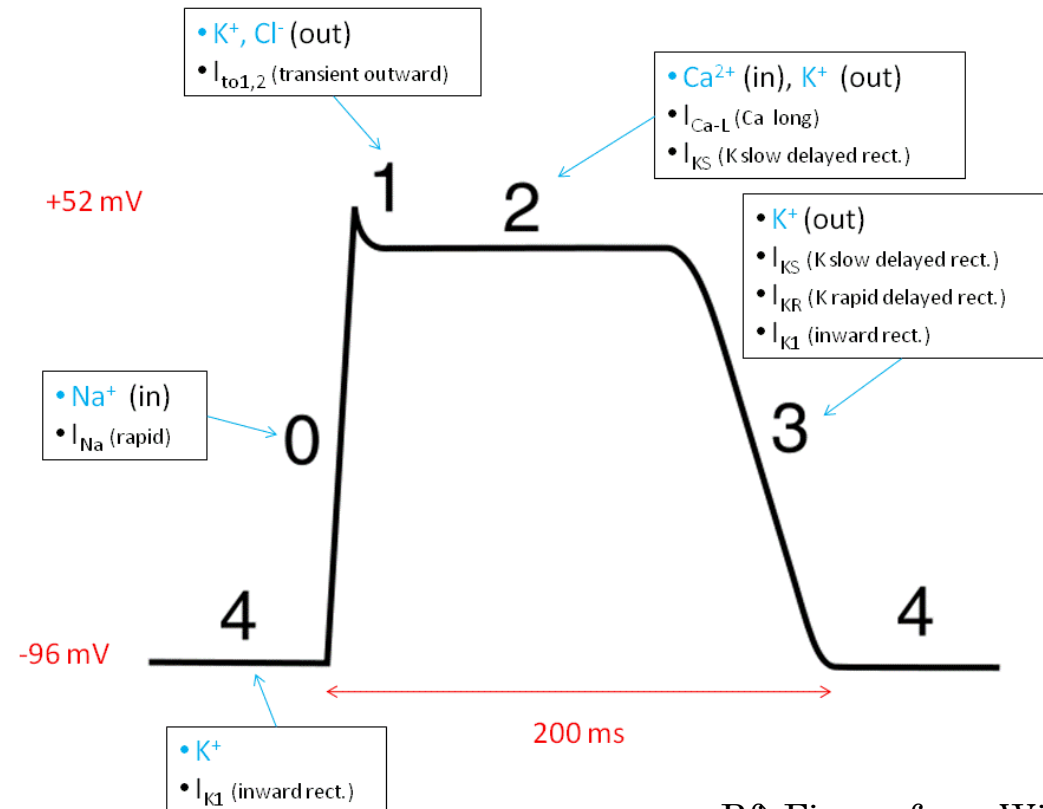
- ICH S7B
- ICH E14 AND Q&A 5.1 OR 6.1
- ICH S7B Q&A 1.1
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# ICH S7B GUIDELINE

## 1.2 Back ground

- The human ventricular action potential consists of five sequential phases (0 to 4):



Rf) Figure from WikiMedia Commons, Basic cardiac action potential

# ICH S7B GUIDELINE

## 1.2 Back ground

- The rapidly and slowly activating components of the **delayed rectifier potassium current,  $I_{Kr}$  (hERG) and  $I_{Ks}$** , seem to have the **most influential role** in determining the duration of the **action potential** and thus the **QT interval**.
- **The most common mechanism** of QT interval prolongation by pharmaceuticals is inhibition of the delayed rectifier potassium channel that is **responsible for  $I_{Kr}$  (hERG)**.

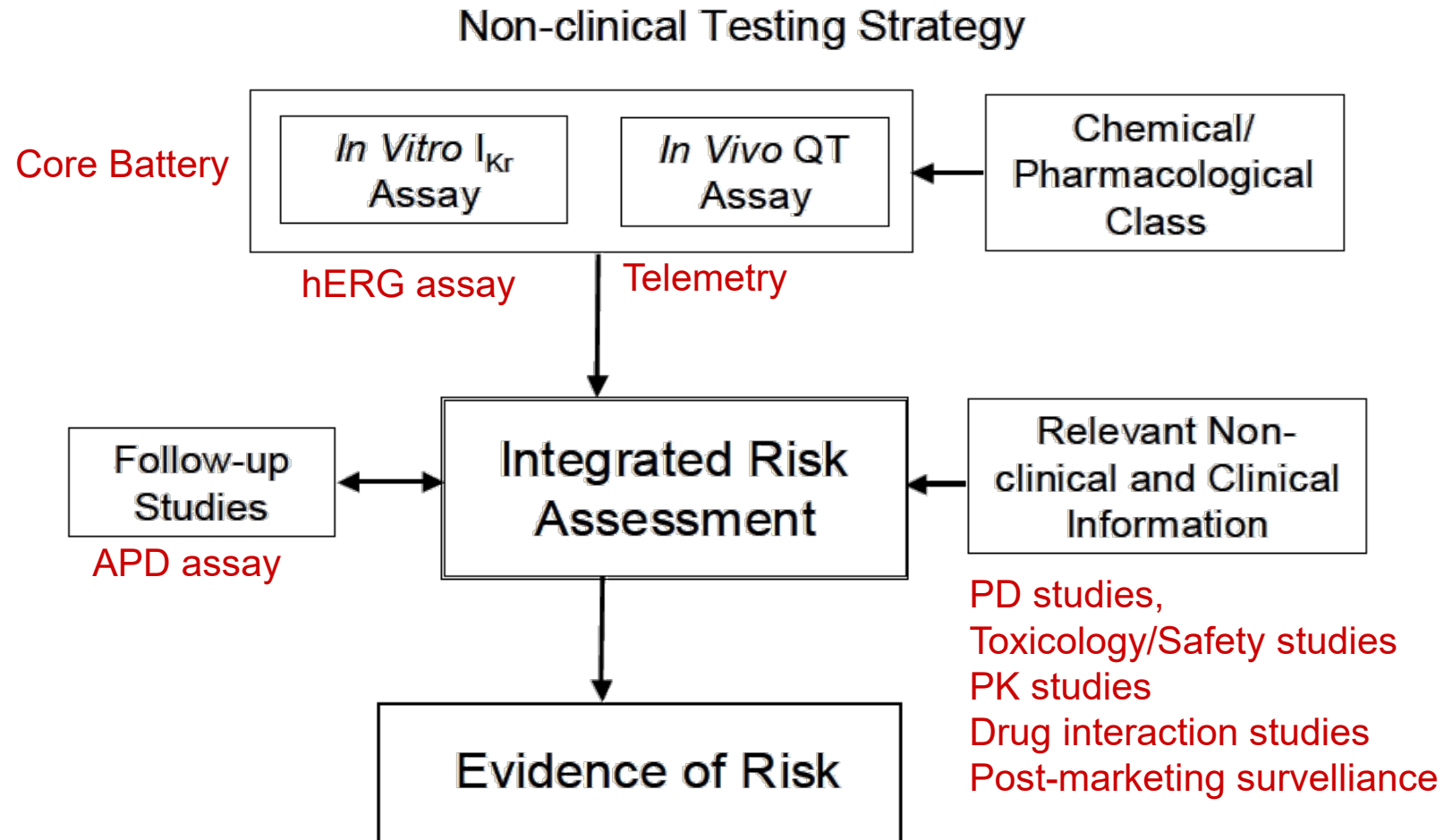
# ICH S7B GUIDELINE

## 2.2 Considerations for Selection and Design of Studies

- **Non-clinical methodologies** can address the following:
  - Ionic currents measurement ( $I_{kr}$ ,  $I_{Na}$ ,  $I_{Ca}$  and so on )
  - Action potential parameters measurement; (APD assay)
  - ECG parameters measured in animals (**Telemetry study**)
  - Proarrhythmic effects measured in isolated cardiac preparations or animals.

## 2.3 Non-clinical Testing Strategy

*The Non-Clinical Evaluation of the Potential for Delayed Ventricular Repolarization (QT Interval Prolongation) by Human Pharmaceuticals*



# ICH S7B GUIDELINE

## 3.1 Considerations for Test system

### 3.1.2 *In vitro* Electrophysiology studies

- *In vitro* electrophysiology studies employ either **single cell (e.g., heterologous expression systems, disaggregated cardiomyocytes)** or **multicellular (e.g., Purkinje fiber; papillary muscle; trabeculae; perfused myocardium; intact heart)** preparations.
- **Heterologous expression systems**, where human ion channel protein(s) are expressed in **noncardiac cell lines**, are used to assess the effects of a test substance on a **specific ion channel** ( $I_{Kr}$ ,  $I_{Na}$ ,  $I_{Ca}$ . And so on)
- Multicellular preparations are stable test systems to study **action potential duration (APD)**. The analysis of parameters for each phase of the action potential such as  $V_{max}$  for phase 0 ( $I_{Na}$ ),  $APD_{30}$  or  $APD_{40}$  for phase 2 ( $I_{Ca}$ ) and “triangulation” for phase 3 ( $I_K$ ) can be useful to investigate the effects on specific channels responsible for these phases.
- **The ionic mechanisms of repolarization in adult rats and mice differ from larger species**, including humans (the primary ion currents controlling repolarization in adult rats and mice is  $I^{to}$ ); therefore, use of tissues from these species is **not considered appropriate**.

# ICH S7B GUIDELINE

## 3.1 Considerations for Test system

### 3.1.2 *In vitro* Electrophysiology studies

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

# ICH S7B GUIDELINE

## 3.1 Considerations for Test system

### 3.1.2 *In vitro* Electrophysiology studies

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

# ICH S7B GUIDELINE

## 3.1 Considerations for Test system

### 3.1.2 *In vivo* Electrophysiology studies

- The QT interval of the ECG is the most commonly used endpoint to gauge effects of a test substance on ventricular repolarization.
- Additional safety parameters of interest, including blood pressure, heart rate, PR interval, QRS duration, and arrhythmias, can be assessed simultaneously.
  - The QT interval and heart rate have an inverse, non-linear relationship, which varies among species and between animals within a species. Thus, a change in heart rate exerts an effect on QT interval, which can confound the assessment of the effect of the test substance on ventricular repolarization and the QT interval.
  - When the effects are due to a test substance, the most common approach is to correct the QT interval for heart rate (QTc) using formulae such as Bazett or Fridericia. An analysis of QT/RR relationship, including correction of the QT interval using formulae for individual animals, may be more appropriate.

# ICH S7B GUIDELINE

## 3.1 Considerations for Test system

### 3.1.2 *In vivo* Electrophysiology studies

- The **dose range** should **include and exceed the anticipated human exposure**. The dose range can be **limited by animal intolerance** to the test substance, e.g., emesis, tremor, or hyperactivity.
- Laboratory animal species used for *in vivo* electrophysiology studies include **dog, monkey, swine, rabbit, ferret, and guinea pig**. **The ionic mechanisms of repolarization in adult rats and mice differ from larger species**, including humans (the primary ion currents controlling repolarization in adult rats and mice is  $I^{to}$ ); therefore, use of these species is not considered appropriate.

# ICH E14 GUIDELINE

INTERNATIONAL CONFERENCE ON HARMONISATION OF TECHNICAL  
REQUIREMENTS FOR REGISTRATION OF PHARMACEUTICALS FOR HUMAN USE

ICH HARMONISED TRIPARTITE GUIDELINE

**THE CLINICAL EVALUATION OF QT/QTc INTERVAL  
PROLONGATION AND PROARRHYTHMIC POTENTIAL FOR NON-  
ANTIARRHYTHMIC DRUGS**

**E14**

*Current Step 4 version*  
dated 12 May 2005

# ICH E14 GUIDELINE

## 1.3 Scope

- The recommendations contained in this document are generally applicable to **new drugs having systemic bioavailability**, but may not apply to products with highly localized distribution and those administered topically and not absorbed.

## 2.1 Overall Approaches to Evaluating Drug Effects on QT/QTc Interval

- Drugs are expected to receive a clinical electrocardiographic evaluation, **beginning early in clinical development**, typically including a single trial dedicated to evaluating their effect on cardiac repolarization (**thorough QT/QTc study**).
- **Factors that could reduce the need** for such a study include the **inability to conduct the study in healthy volunteers** or patients, how the drug is studied and used (e.g., administered under continuous monitoring), **as well as nonclinical data**.

# ICH E14 GUIDELINE

## 2.2 The 'Thorough QT/QTc Study'

- The threshold level of regulatory concern, discussed further below, is around 5 ms as evidenced by an upper bound of the 95% confidence interval around the mean effect on QTc of 10 ms.
- The results of the 'thorough QT/QTc study' will influence the amount of information collected in later stages of development:
  - A negative 'thorough QT/QTc study' will almost always allow the collection of on-therapy ECGs in accordance with the current practices in each therapeutic area to constitute sufficient evaluation during subsequent stages of drug development;
  - A positive 'thorough QT/QTc study' will almost always call for an expanded ECG safety evaluation during later stages of drug development.

# ICH E14 GUIDELINE

## 2.2 The 'Thorough QT/QTc Study'

### 2.2.5 Alternative Strategies to Assess QT/QTc Interval Effects

- Alternatives to the use of the 'thorough QT/QTc study' are **under active investigation**. Examples include evaluating the relationship between concentration and QT/QTc effects or more intensively evaluating ECGs, based on data collected during early phase clinical studies.

# ICH E14 GUIDELINE

## 2.4 Clinical Development When the 'Thorough QT/QTc Study' Can not be Performed in Healthy Volunteers (*special case*)

- There are some drugs that cannot be studied in a 'thorough QT/QTc study' in healthy volunteers due to **safety or tolerability concerns** (e.g., **cytotoxic cancer drugs**). In such cases, the 'thorough QT/QTc study' can often be **conducted in patient populations**. When this is not possible, the importance of detecting and modifying this safety risk means that other ways of detecting effects on the QT/QTc interval need to be developed.
- These might include the collection of ECGs at multiple timepoints under tightly controlled settings that target a broad range of doses early in development.
  - **(E14 Q&A (R3); Q6.1** The ICH E14 Guideline states that in QT study might not be feasible. In such cases what other methods should be used for evaluation of QT/QTc and proarrhythmic potential ?)

# ICH E14 GUIDELINE

## 3. Analysis of ECG data from Clinical Trials

### 3.2 Analysis of QT/QTc Interval Data

#### 3.2.3 Analysis of Relationship Between Drug Exposure and QT/QTc Interval Changes

- Establishing the relationship of drug concentrations to changes in QT/QTc interval may provide **additional information to assist the planning and interpretation of studies assessing cardiac repolarization**. This area is **under active investigation**.

→ **(E14 Q&A(R3) 5.1;** 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 ?

# ICH E14 Q&A

## 5. Use of Concentration Response Modeling of QTc Data

**Q 5.1;** 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 ?)

**A 5.1;** Concentration-response analysis, in which **all relevant data across all doses are used to characterize** the potential for a drug to influence QTc, can serve as an alternative to the by-time-point analysis or intersection-union test as the primary basis for decisions to **classify the risk of a drug**. In either case this result is an important component of **the totality of evidence assessment of the risk** of QT prolongation.

# ICH E14 Q&A

## 5. Use of Concentration Response Modeling of QTc Data

### A 5.1; Dose and exposure definitions

- **Therapeutic dose:** dose evaluated in Phase 3 trial or recommended in product labeling
- **Clinical exposure:** **mean steady state maximum concentration** ( $C_{\max, ss}$ ) associated with the maximum therapeutic dose
- **High clinical exposure (HCE):** exposure ( $C_{\max, ss}$ ) 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  $C_{\max, ss}$
- **Supratherapeutic dose:** dose that provides exposures (mean  $C_{\max}$ ) exceeding the high clinical scenario

# ICH E14 Q&A

## 5. Use of Concentration Response Modeling of QTc Data

### **A 5.1; Important considerations**

1. Data can be acquired from first-in-human (FIH) studies, multiple ascending dose (MAD) studies, or other studies provided that the concentrations that can be safely achieved are well above the exposure at the maximum therapeutic dose at steady-state, and reflect high clinical exposure scenario situations such as drug-drug and drug-food interactions, organ dysfunction, and/or genetically impaired metabolism. Whenever possible, sponsors are encouraged to explore a wide dose range in early phase studies to enable characterization of effects at high concentrations.
2. Efficient concentration-response analysis using data acquired in studies with other purposes requires as much quality control as is needed for a dedicated study. This includes robust, high-quality electrocardiogram (ECG) recording and analysis sufficient to support a valid assay for ECG intervals (see ICH E14 and Q&A #1).
3. If there is an intention to pool ECG interval data from multiple studies, it is important to test for heterogeneity. Pooling of studies that were not planned for this purpose can produce bias. This potential should be critically discussed in the analysis plan.

# ICH E14 Q&A

## 5. Use of Concentration Response Modeling of QTc Data

### A 5.1; Important considerations

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;

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). **<Nonclinical double negative + covering high clinical exposure>**

# ICH E14 Q&A

## 5. Use of Concentration Response Modeling of QTc Data

### **A 5.1; Decision-making**

Both the intersection-union test and the concentration-response analysis can estimate the maximum effect of a drug treatment on the QTc interval, but they are not used to test the same hypothesis. As mentioned above, inspection of **the time course of QT prolongation is important**. However, hypothesis testing based on a by-time point analysis (intersection-union test or point estimate and confidence intervals) is inappropriate in studies designed for a concentration-response analysis, if not powered to assess the magnitude of QT prolongation for each time point. When using a concentration-response analysis as the primary basis for decisions to classify the risk of a drug, the upper bound of the two-sided 90% confidence interval for the QTc effect of a drug treatment as estimated by exposure-response analysis **should be <10 ms at the highest clinically relevant exposure to conclude that an expanded ECG safety evaluation during later stages of drug development is not needed**. (See ICH E14, Section 2.2.4 and Q&A #7).

# ICH E14 Q&A

## 6. Special Cases

**Q 6.1;** The ICH E14 Guideline states that 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?

**A 6.1;** An **integrated nonclinical and clinical QT/QTc risk assessment** can be particularly **valuable** when a thorough QT study or concentration-QTc analysis meeting similar quality control as needed for a dedicated study as described in 5.1 is not feasible. This situation can arise under scenarios where a **placebo-controlled comparison is not possible**; safety considerations preclude administering suprathreshold doses to obtain high clinical exposures and/or **safety or tolerability** prohibit the use of the product in healthy participants. The design elements that include placebo and healthy participant dosing assist in decreasing variability, but their absence does not preclude interpretation.

# ICH E14 Q&A

## 6. Special Cases

### A 6.1

The integrated nonclinical and clinical QT/QTc risk assessment should include:

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

In situations where it is not possible to evaluate the QT/QTc effects at high clinical exposure, it is particularly important that the nonclinical *in vivo* studies are conducted at exposures covering the high clinical exposure (see ICH E14 Q&A 5.1 for definition of high clinical exposure).

# ICH NEW S7B Q&A

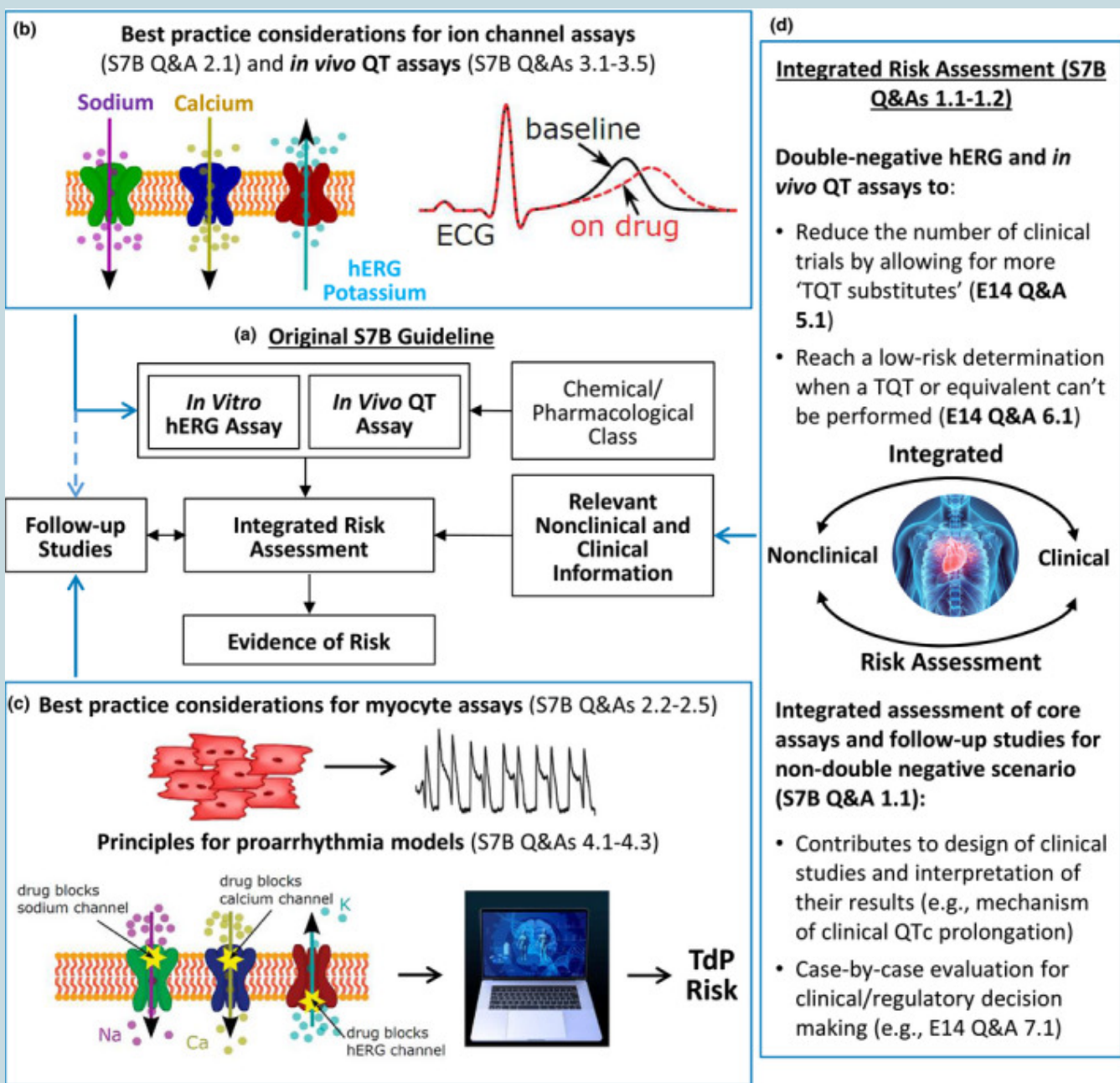
## 1. Integrated Risk Assessment

**Q 1.1** The 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?

**A 1.1** The following are points to consider when using *in vitro* **IKr/hERG** data and **in vivo QT** data in combination with clinical QT data as part of an **integrated risk assessment for situations described in ICH E14 Q&As 5.1 & 6.1.**

1. To predict whether or not the hERG block poses a risk of interfering with ventricular repolarization or TdP, evaluation of the **hERG safety margin based on results of a best practice assay** (see S7B Q&As 1.2 and 2.1) is **recommended.**

ICH S7A Section 2.6 describes considerations for when human metabolite(s) should be assessed with in vitro systems. In these cases, the **metabolite's hERG safety margin** should also be evaluated.



Rf) Figure from Clin Pharmacol Ther. (2021 Feb) 109(2):319-333 and ICH E14/S7B Q&A Training Materials, ICH (2022)

# ICH NEW S7B Q&A

## 1. Integrated Risk Assessment

**A 1.1** The following are points to consider when using in vitro IKr/hERG data and in vivo QT data in combination with clinical QT data as part of an **integrated risk assessment for situations described in ICH E14 Q&As 5.1 & 6.1.**

2. In the *in vivo study*, the effects on the QTc interval should be assessed at exposures that **cover the anticipated high clinical exposure scenario.** The adequacy of exposure to any major human metabolites should be determined (see ICH S7A Sections 2.3.3.2 & 2.6, and S7B Q&A 3.5). In addition, if the assay is to be used as part of an integrated clinical and nonclinical risk assessment for situations where a conventional thorough QT study is not feasible as described in **ICH E14 Q&A 6.1 (special case)**, the *in vivo study* should have **sufficient sensitivity to detect a QTc prolongation effect** of a magnitude similar to dedicated clinical QT studies (see ICH S7B Q&A 3.4). This additional consideration (sensitivity similar to dedicated clinical QT studies) does **not apply** to decision making prior to First-in-Human studies or under **ICH E14 Q&A 5.1.**

# ICH NEW S7B Q&A

## 2. Best Practice Considerations for *In vitro* Studies

**Q 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?

**A 2.1** The following “best practice” aspects should be considered when sponsors are using IKr/hERG data to support interpretation of clinical QT data in specific scenarios as described in S7B Q&As 1.1 & 1.2 (hERG safety margins) and ICH E14 Q&As 5.1 & 6.1, and when using CaV1.2 and NaV1.5 to support a proarrhythmia assessment (ICH S7B Q&As 1.1).

It is **not the intent** of these Q&As to make specific recommendations for a **sponsor’s screening activities** or for **all IKr/hERG assays to support first administration in humans**.

# ICH NEW S7B Q&A

## 2. Best Practice Considerations for *In vitro* Studies

**A 2.1** Several experimental factors are known to influence the potency of drug effects on cardiac ionic currents. These include the voltage protocols used to evoke specific ionic currents, experimental conditions (such as recording temperature, composition of solutions, manual vs. automated assay systems), data acceptance criteria, and data analysis methods employed.

1. **Recording temperature:** The effects of some drugs are temperature-sensitive and there is currently no method to predict which molecules exhibit temperature-dependent effects or the magnitude of these effects. Thus, patch clamp experiments on cells overexpressing cardiac ion channels, including hERG, CaV1.2, and NaV1.5, should be performed at near **physiological temperature (35–37 °C)**.

2. **Voltage protocol:** The voltage protocols used to evoke ionic currents should approximate the appropriate elements of a ventricular action potential and be repeated at frequencies that are sufficient to minimize the possibility of missing the effects of a test drug at physiologically relevant heart rates.

# ICH NEW S7B Q&A

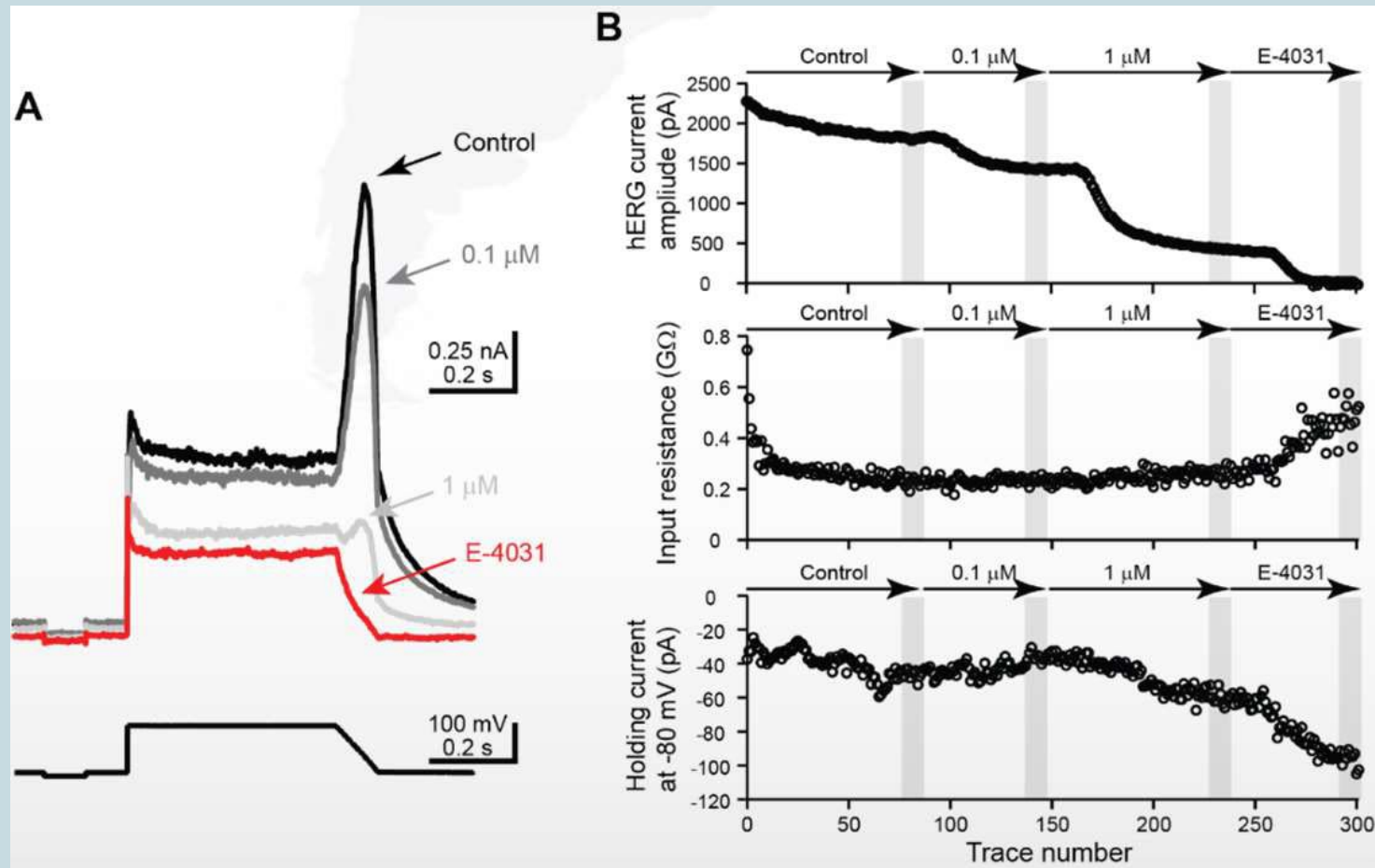
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3. **Recording quality: Seal resistance** should be high enough so that the **leak conductance** at all voltages specified by the voltage protocol and series resistance do not compromise voltage control. The extent of series resistance compensation applied to optimize voltage control should be noted. **Stability of the ionic current** should be demonstrated with **baseline recordings** (prior to drug application) of sufficient duration to characterize drug-independent changes (such as current rundown).

**The time course of drug effects should be monitored** until steady state effect is obtained, and each cell can be exposed to one or more drug concentrations as long as **cell health and recording quality remain stable**.

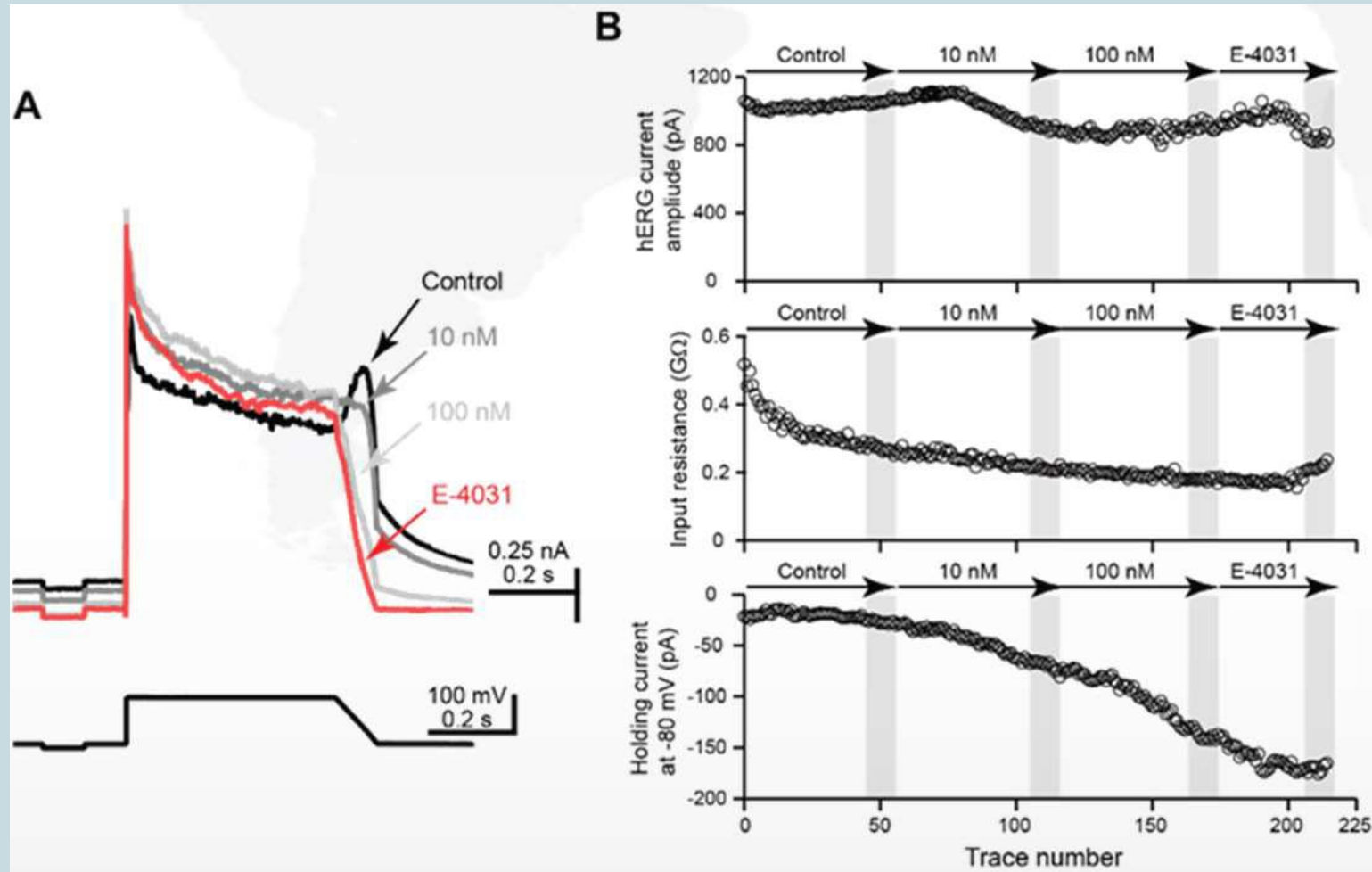
## ICH S7B Q&A 2.1 *In vitro* Best Practice, Recording Quality



Example of recording quality for individual cell

Rf) Figure from ICH E14/S7B Q&A Training Materials (March 2022) ICH

## ICH S7B Q&A 2.1 *In vitro* Best Practice, Recording Quality



Example of **inadequate** recording quality

Rf) Figure from ICH E14/S7B Q&A Training Materials (March 2022) ICH

# ICH NEW S7B Q&A

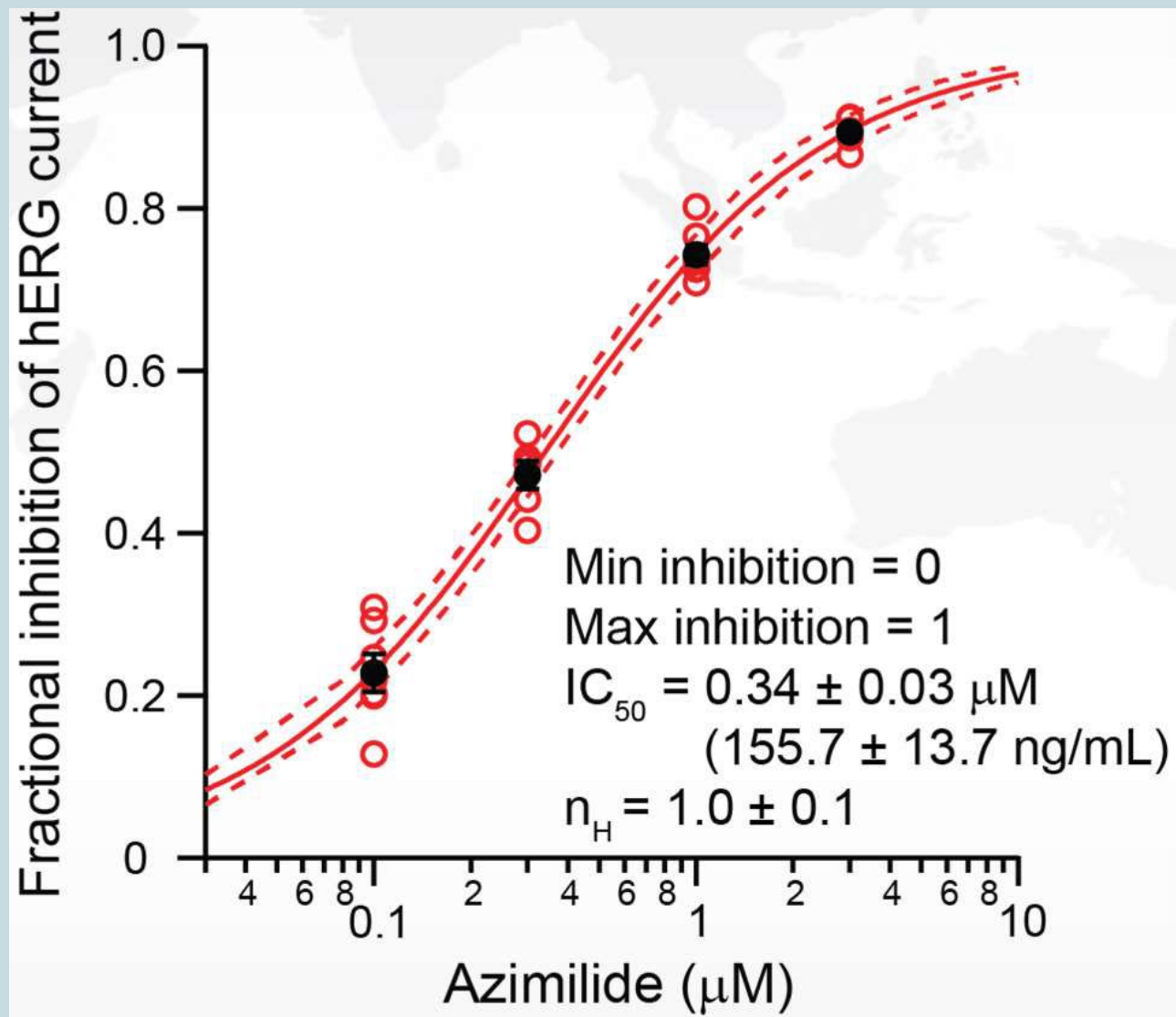
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4. **Primary endpoint measures:** The primary derived endpoints are inhibitory concentration such as the **IC<sub>50</sub> value** (reported in both micromolar and ng/mL units) and **Hill coefficient**.

**If 50% current inhibition could not be achieved**, a justification of the highest concentration tested should be provided together with the relation **of this concentration to therapeutic free and total drug levels**.

## ICH S7B Q&A 2.1 *In vitro* Best Practice, Primary endpoint



Plot concentration-inhibition graph using individual data points (red symbols) and mean  $\pm$  SEM (black symbols/error bars)

Half inhibitory concentration ( $IC_{50}$ ), Hill coefficient ( $n_H$ ), and uncertainty estimation (95% confidence interval or CI) should be reported.

# ICH NEW S7B Q&A

## 2. Best Practice Considerations for *In vitro* Studies

**A 2.1 Several experimental factors** are known to influence the potency of drug effects on cardiac ionic currents. These include the **voltage protocols** used to evoke specific ionic currents, experimental conditions (such as **recording temperature**, **composition of solutions**, manual vs. automated assay systems), data acceptance criteria, and data analysis methods employed.

5. **Data summary: Inhibition at each drug concentration for each cell** should be provided, along with the mean values of  $IC_{50}$  and Hill coefficient (and appropriate measures of data variability). To demonstrate **recording quality**, the study report should also contain **time-course plots of current amplitude, input resistance, holding current for individual cells** in control condition followed by drug application, and drug equilibration.

## ICH S7B Q&A 2.1 *In vitro* Best Practice, Data summary

Nominal [Azimilide] ( $\mu\text{M}$ )	Fractional inhibition													n	Mean	SD	sem
	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				
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

Example of Table. Fractional inhibition for each cell

Rf) Table from ICH E14/S7B Q&A Training Materials (March 2022) ICH

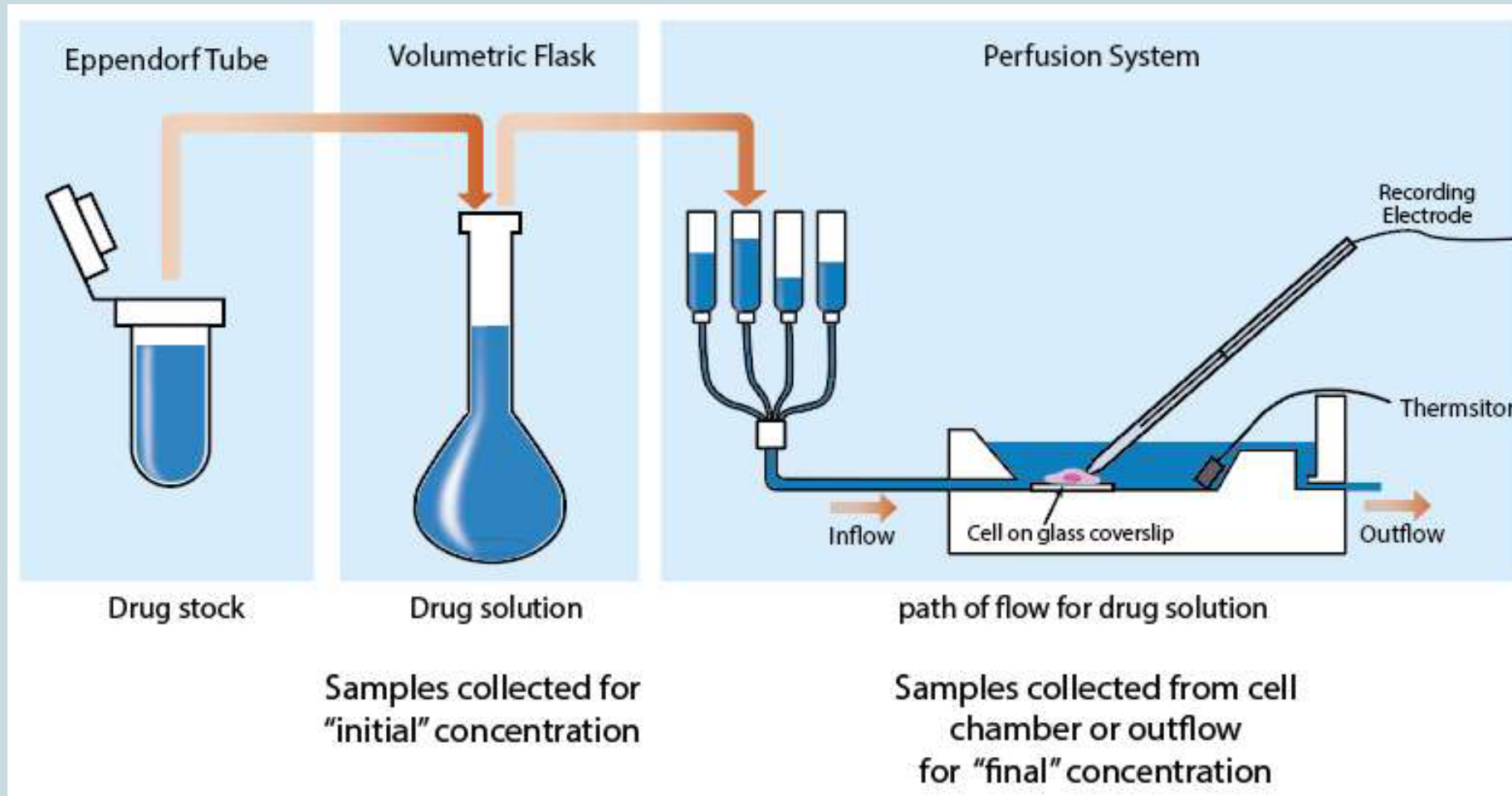
# ICH NEW S7B Q&A

## 2. Best Practice Considerations for *In vitro* Studies

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6. **Concentration verification**: The concentration of test compound to which the cells were exposed should be verified by applying a **validated analytical method** to the solution collected **from the cell chamber**. **Both nominal and measured concentrations should be reported**. If the nominal and measured concentrations differ significantly from each other, measured concentrations should be used to construct the concentration-response relationship to estimate  $IC_{50}$  and Hill coefficient.

## ICH S7B Q&A 2.1 *In vitro* Best Practice, Concentration verification



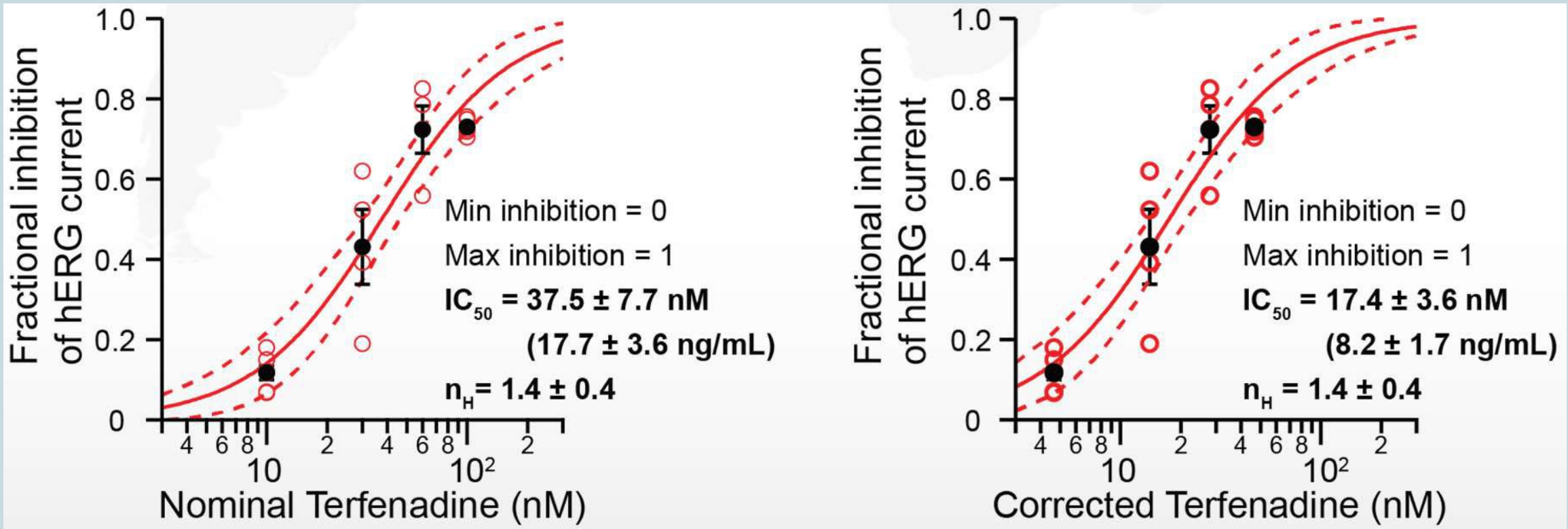
## ICH S7B Q&A 2.1 *In vitro* Best Practice, Concentration verification

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		
				<b>Mean % loss</b>	53.4

Example of Table. Initial and Final concentration and % Loss

Rf) Figure from ICH E14/S7B Q&A Training Materials (March 2022) ICH

# ICH S7B Q&A 2.1 *In vitro* Best Practice, Primary endpoint



Example of the plot of concentration vs fractional inhibition with corrected concentration

Rf) Figure from ICH E14/S7B Q&A Training Materials (March 2022) ICH

# ICH NEW S7B Q&A

## 2. Best Practice Considerations for *In vitro* Studies

**A 2.1 Several experimental factors** are known to influence the potency of drug effects on cardiac ionic currents. These include the **voltage protocols** used to evoke specific ionic currents, experimental conditions (such as **recording temperature**, **composition of solutions**, manual vs. automated assay systems), data acceptance criteria, and data analysis methods employed.

7. **Positive and negative controls: The positive control drug should be one of the “reference drugs”** referred to in Q&A 1.2. The positive control drug should be tested using sufficient replicates and **two or more concentrations achieving 20-80% block**, to demonstrate consistency and reproducibility with the reference drug data. If positive control data fall outside the range of expected values, then the study is inconclusive, and it would not be recommended that the data be used to support the purposes outlined in ICH E14 Q&As 5.1 and 6.1. Vehicle (negative) controls should be included in the experiments. The vehicle should include all non-compound materials in the test article solution such as solubilizing agents and preservatives.

# ICH NEW S7B Q&A

## 3. Best Practice Considerations for *In vivo* Studies

**Q 3.1** What are **best practice** considerations for **species selection and general design** of the (standard) *in vivo* QT study?

**A 3.1** It is preferable to use the **same animal species in the safety pharmacology and non-rodent toxicity studies** to facilitate understanding of the possible relationship between adverse cardiovascular pharmacodynamic effects and structural effects on the heart, and to obtain complementary information on systemic exposure level (toxicokinetics).

While it is **customary** to use **conscious freely moving telemeterized animals** for the *in vivo* QT studies, the choice of alternative model approaches (e.g., anesthetized or paced animals) might be justified in certain circumstances to achieve adequate exposures or to overcome specific compound-related challenges (e.g., changes in heart rate, tolerability, or bioavailability limitations in conscious animals).

# ICH NEW S7B Q&A

## 3. Best Practice Considerations for *In vivo* Studies

**Q 3.2** What should be considered for **exposure assessment** during the *in vivo* QT study?

**A 3.2** The ICH S7B guideline states that **drug exposures should include and exceed anticipated therapeutic concentrations**. If the *in vivo* QT data are to be used as part of an **integrated risk assessment** for situations described in ICH E14 Q&As 5.1 & 6.1, the exposure should cover the **anticipated high clinical exposure (HCE) scenario** (see S7B Q&A 1.1).

**An assessment of exposure in the same animals used for the pharmacodynamic assessment is encouraged**. Sampling should take place at relevant timepoints and in a manner that limits interference with the pharmacodynamic effects. This could be done by sampling complete pharmacokinetic profiles in **the same animals on a separate day** after an adequate washout **or different animals**.

At least one pharmacokinetic sample should be obtained during the pharmacodynamic assessment day to demonstrate consistency with the full pharmacokinetic profiles. In certain cases, **the analysis of QTc interval together with adequate pharmacokinetic sampling** makes it possible to perform **dedicated exposure-response modeling similar to concentration- QT analysis for clinical QT studies**.

This can be helpful when the study should be powered to detect an effect similar to dedicated QT studies in humans (e.g., when using *in vivo* QT data as part of an **integrated nonclinical and clinical risk assessment as described in ICH E14 Q&A 6.1**) as it can reduce the number of animals in accordance with the 3R (reduce/refine/replace) principles.

# ICH NEW S7B Q&A

## 3. Best Practice Considerations for *In vivo* Studies

**Q 3.3** How should the sensitivity of the assay be evaluated?

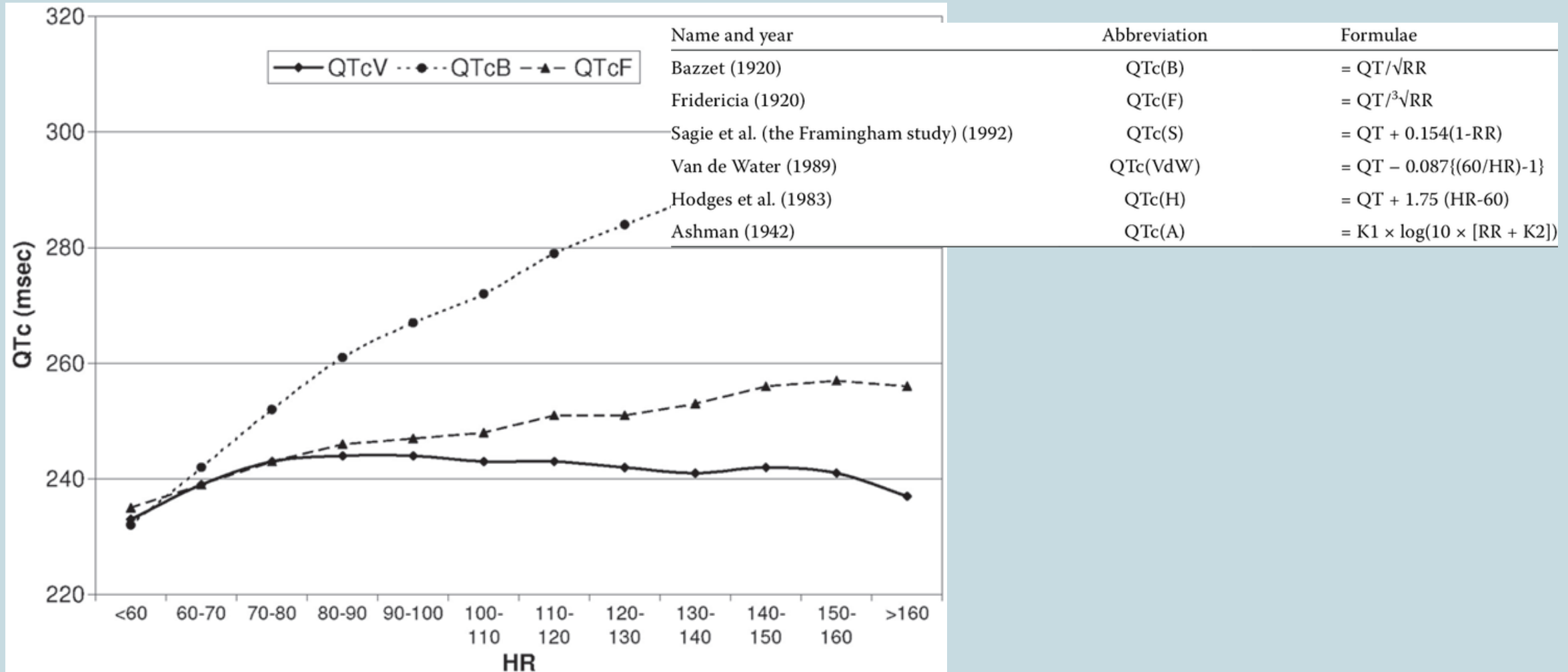
**A 3.3** Optimally, the sponsor should demonstrate **the independence of QTc to RR intervals** observed in the study through QTc versus RR plots accompanied by additional information (e.g., number of matched QTc-RR pairs, correlation metric, 95% confidence intervals, p-values).

In certain cases, **individual QT correction based** on QT-RR relationship is a preferred method as it is more accurate and sensitive than the general methods such as Bazett, Fridericia, or Van de Water **when the test drugs affect heart rate**.

<Example of **individual** QT correction>

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

# ICH S7B Q&A 3.3 *In vivo* Best Practice, Heart Rate Correction Method



Comparison of Van de Water’s, Bazett’s, and Fridericia’s QT interval correction formulas (data from 24,882 ECG recordings from 66 beagle dogs)..

Rf) Figure from Journal of Cardiovascular Toxicology. Vol. 6. 51-62 (2006) and ICH E14/S7B Q&A Training Materials (March 2022) ICH

# ICH NEW S7B Q&A

## 3. Best Practice Considerations for *In vivo* Studies

**Q 3.4** What information is needed to support the choice of **heart rate correction method** in an *in vivo* QT assay?

**A 3.4** The test system used for an *in vivo* QT assay should provide a robust response. **Assay sensitivity** of relevant functional endpoints should be evaluated and reported to enable data interpretation (in supporting initiating first-in-human studies and/or an **integrated nonclinical and clinical integrated risk assessment to be applied under the scenarios in ICH E14 Q&As 5.1 or 6.1**) and contextualization.

**Demonstration of assay sensitivity** can be achieved by defining **minimum detectable differences (MDD)** and **testing the effects of positive controls**. Statistical power calculations could also be provided from historical data from the same laboratory using the identical protocol. If historical positive control data are utilized to justify assay sensitivity or statistical power is calculated **from historical control data**, then the variance of the present data should be consistent with that seen historically.

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

# ICH NEW S7B Q&A

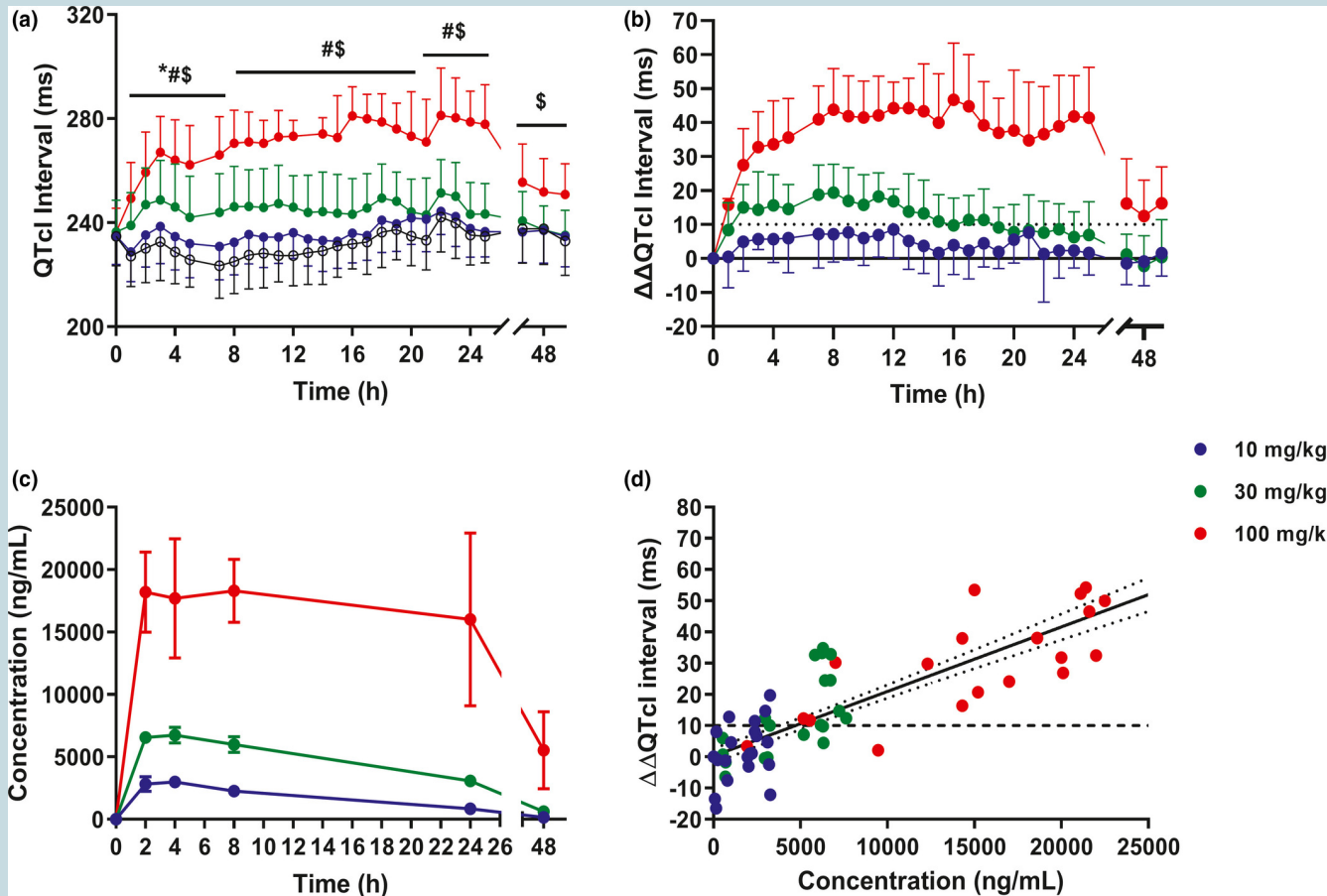
## 3. Best Practice Considerations for *In vivo* Studies

**Q 3.4** What information is needed to support the choice of **heart rate correction method** in an *in vivo* QT assay?

**A 3.4** The following hypothetical example is offered for consideration with recognition that 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.

- *Hypothetical Example*: The minimal detectable difference (**MDD**) might be **5 milliseconds** if **drug exposure** in the animal study only **covers the high clinical exposure (HCE)** but might be higher if a larger multiple of high clinical exposure is achieved (e.g., 10 milliseconds if 3X high clinical exposure (HCE) is achieved; or a higher **QTc threshold** if an even larger multiple is achieved).

# ICH S7B Q&A 3.4 *In vivo* Best Practice, Use of a positive control to demonstrate Sensitivity, PD, PK, Data summary



**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.

Rf) Figure from Clinical Translational Sci, Vol. 14, 2379-2390 (2021) and ICH E14/S7B Q&A Training Materials (March 2022) ICH

Moxifloxacin(**positive control**) was tested to demonstrate QTc sensitivity with by time-response and concentration-QTc analysis

- The low dose (10 mg/kg) of moxifloxacin increased QTc intervals by 5.9 ms ( $p < 0.05$ ) at  $C_{max}$  of 2980 ng/ml (total).
- Linear-regression demonstrated clinically relevant **detection sensitivity**. A 10 ms change was estimated at a total plasma concentration of 4627 ng/ml.
- Free concentrations of moxifloxacin that produce a 10 ms QTc change were 2 to 2.5-fold larger than human thorough QT study data.

# ICH NEW S7B Q&A

## 3. Best Practice Considerations for *In vivo* Studies

**Q 3.5** What are the recommended conventions for presenting the **pharmacodynamic and pharmacokinetic results** of an *in vivo* QT assay?

**A 3.5** To facilitate the regulatory review of an *in vivo* QT assay, the following are general recommendations.

### - Pharmacodynamic Content

- Summary tables and figures showing **absolute mean values, mean percent change from baseline, confidence intervals, and p-values for changes from baseline** and vehicle control should be included.
- If study results are being used to **support ICH E14 Q&A 6.1**, then data from **positive controls should be included** or appended.

If historical positive controls are used, then **the variance of the present data should be consistent with that seen historically**, which can be demonstrated by reporting minimal detectable differences (MDD) with by time analysis.

If **concentration-QT<sub>c</sub> modeling** is performed, reporting should follow **similar principles as for human concentration-QT<sub>c</sub> modeling** (see ICH E14 Q&A 5.1).

# ICH NEW S7B Q&A

## 3. Best Practice Considerations for *In vivo* Studies

**Q 3.5** What are the recommended conventions for presenting the **pharmacodynamic and pharmacokinetic results** of an *in vivo* QT assay?

**A 3.5** To facilitate the regulatory review of an *in vivo* QT assay, the following are general recommendations.

### - Pharmacokinetic Content

- Tabulations of summary statistics for  **$C_{\max}$ , AUC, and  $T_{\max}$**  for the parent drug and metabolites along with **plasma concentration vs. time plots** (if sufficient samples have been collected to support their calculation) should be provided.

# SUMMARY

- **The new integrated risk assessment Q&As provide additional recommendations when nonclinical data are used later in clinical development**
- **A Double negative nonclinical assessments (*in vitro* hERG and *in vivo* QT) can be used to support E14 Q&As 5.1 & 6.1**
- ***In vitro* best practice recommendations for patch clamp studies including the core, *in vitro* hERG assay harmonize approaches to enhance data reproducibility**
- ***In vitro* data quality with voltage protocol, recording quality, concentration verification**
- ***In vivo* best practice provide exposure-response modeling for use in revised ICH E14 Q&As 5.1 and 6.1**
- ***In vivo* data quality with MDD, individual QTc, concentration-QTc analysis**

*Thank you for your attention !*