# Best Practice Considerations for *In Vivo* Studies

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

# 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**
- **Over the last 15 years, lessons have been learned on how to best perform and report the results of *in vivo* assays**
- **As there can be some variation in how the studies are performed, the "best practice" Q&As bring attention to certain considerations**
- **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.**

# 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)**
- **Evaluating assay sensitivity (Q&A 3.4)**
- **Presenting the pharmacodynamic (PD) and pharmacokinetics (PK) results (Q&A 3.5)**

- **Reinforce lessons learned from 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 (e.g., dog, monkey, mini-pig)**
  - **Preferable to use same species as non-rodent toxicity studies**
    - Facilitates understanding of potential relationship cardiovascular PD effects and toxicity (abnormal electrolyte, pathological change, etc.)
    - Provides complementary information on exposure level (toxicokinetices)
  - **Conscious freely-moving telemeterized animals are customary**
  - **Alternative model (e.g., anesthetized or paced animal) may be justified**
    - To achieve adequate exposure
    - To overcome drug-related challenges (e.g., heart rate change, tolerability, bioavailability limitation)

# Q&A 3.2: Best Practice Considerations for Drug Exposure Assessment

- Outline of exposure assessment
- Estimate drug exposure level
- Best practice of PK sampling from same or separate animal to avoid interference with PD effect
- Best practice of choosing relevant time points to utilize exposure-response (E-R) modeling
- Example for E-R modeling by the best practice
- Utilize E-R modeling for human safety

# 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* 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**

***See E14 Q&A presentation by Christine Garnett, FDA**

# 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 could 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 or**
  - **By using limited 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: Example Best Practice PK Sampling to Avoid Interference With QT Assessment

## Using same animals

### PD+PK study

| Animal | ECG phase | | PK phase | ..... |
|---|---|---|---|---|
| A | high dose PD | recovery phase | high dose PK sampling | ..... |
| B | mid. dose PD | recovery phase | mid. dose PK sampling | ..... |
| C | low dose PD | recovery phase | low dose PK sampling | ..... |
| D | vehicle ECG | recovery phase | vehicle PK sampling | ..... |

## Using separate animals

| | PD study | | | | PK study |
|---|---|---|---|---|---|
| Animal | ECG phase | | ECG phase | ..... | |
| A | high dose PD limited PK sampling | recovery phase | Vehicle ECG Blood sampling | ..... | |
| B | mid dose PD limited PK sampling | recovery phase | high dose PD limited PK sampling | ..... | Separate animal without telemetry system |
| C | low dose PD limited PK sampling | recovery phase | mid dose PD limited PK sampling | ..... | |
| D | Vehicle ECG Blood sampling | recovery phase | low dose PD limited PK sampling | ..... | |

# 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**

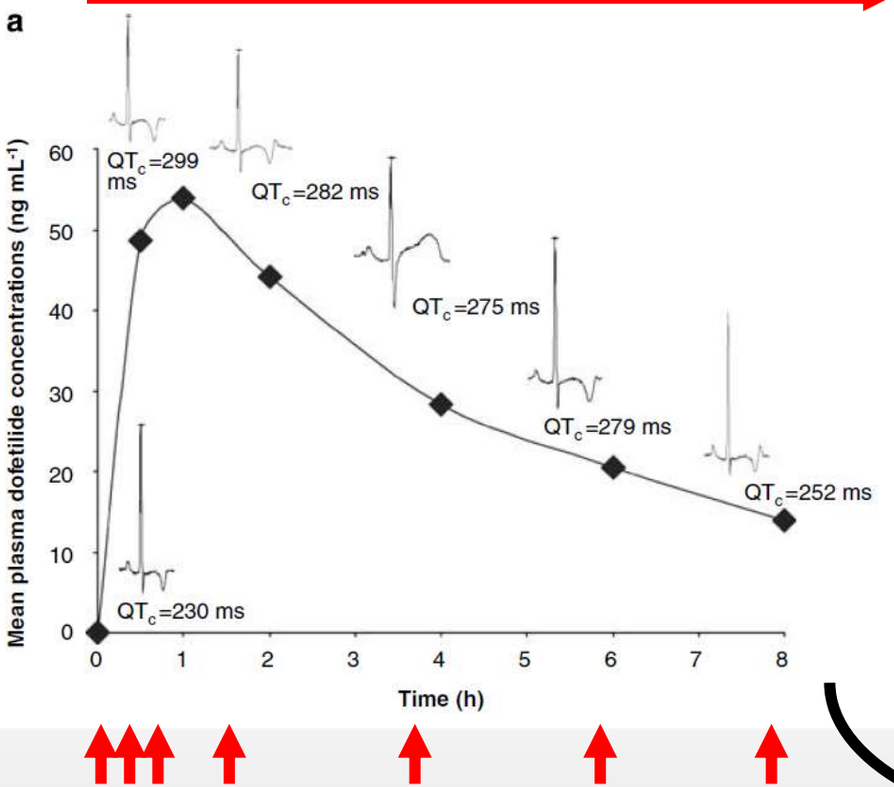
- **This can be helpful when the nonclinical *in vivo* QT assay should be powered to detect an effect similar to dedicated QT studies in humans**
  - **e.g., when using *in vivo* QT data to support clinical decision making under ICH E14 Q&A 6.1**

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

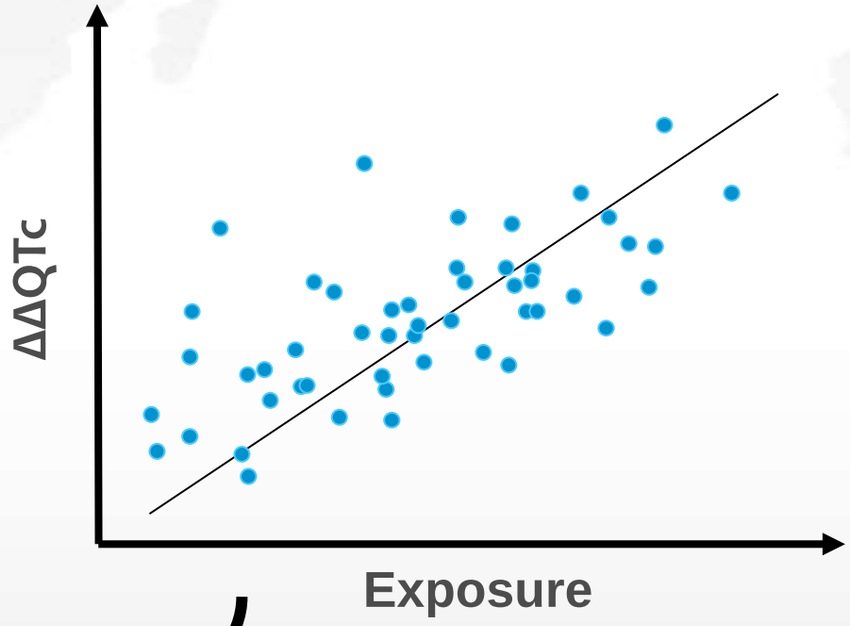
- **Representative references for nonclinical *in vivo* concentration-QTc modeling**
  - **Monkey : Komatsu R *et al*., 2019**
  - **Dog : Dubois VFS *et al*., 2017**

# Q&A 3.2: Example of Best Practice for Adequate PK Sampling to Utilize E-R Analysis

**Telemetry ECG monitoring**



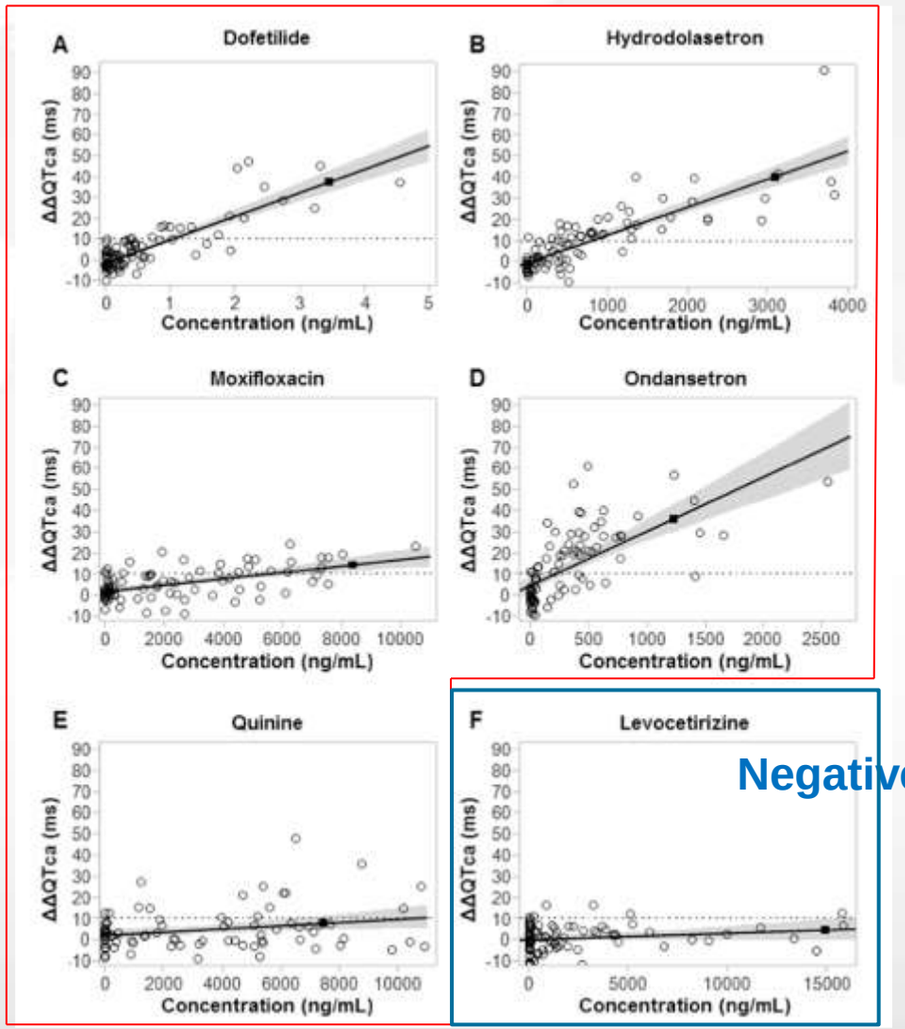

**E-R modeling**




(Haushalter TM., *et al.*, 2008)

# Q&A 3.2: Representative E-R Modeling in Positive and Negative Drug

**Positive drugs**




**Negative drug**

## Species
- Conscious cynomolgus monkeys
- 4 males, 3-6 kg

## ECG
- Freely-moving telemeterized system

## Telemetry data acquisition
- 2h before and 24h after dosing

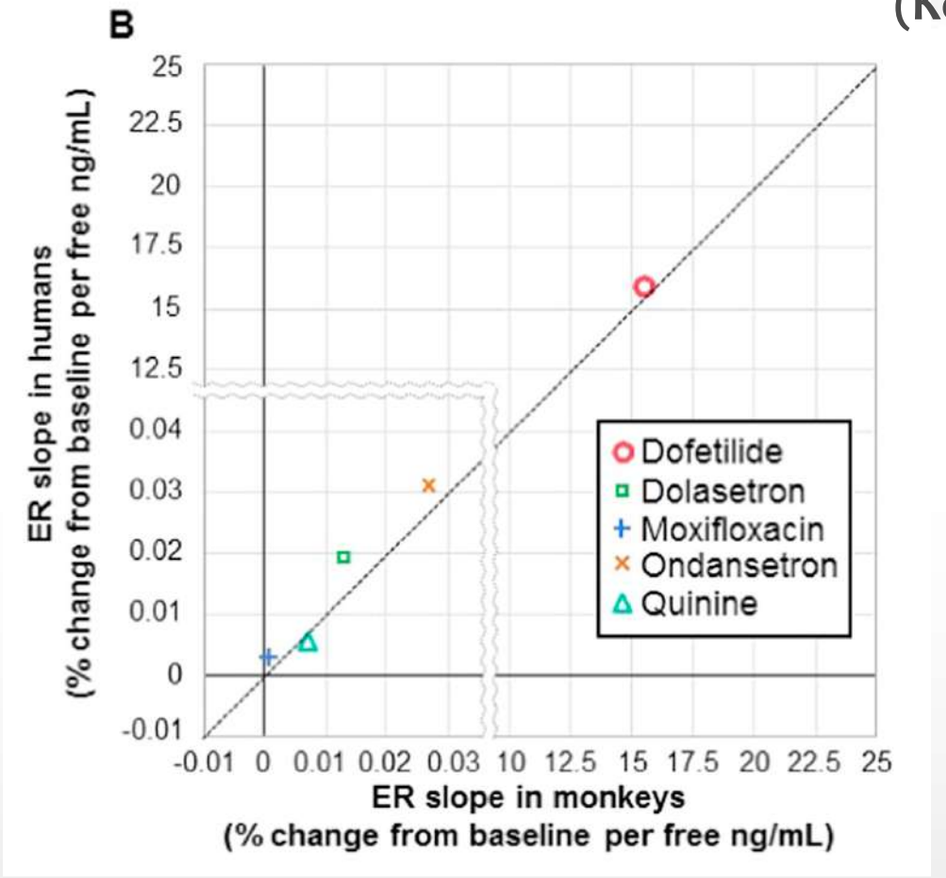
## PK sampling
- 0.5, 1, 2, 4, 8 and 24h from same animals in PD study

## HR correction (see Q&A 3.3)
- Individual rate-corrected QT (QTca)

Study design by J-ICET (Komatsu R., *et al.*, 2019)

# Q&A 3.2: E-R Modeling Results in Power to Detect an Effect Similar to Dedicated QT Studies in Humans

## Nonclinical E-R modeling in comparison to clinical E-R modeling in IQ-CSRC study (Komatsu R *et al.*, 2019)




| QT-positive drug | Animal | Human (IQ-CSRC) # |
|---|---|---|
| | Predicted ΔΔQTca effect at $C_{max}$ (msec) | Predicted ΔΔQTcF effect at $C_{max}$ (msec) |
| Dofetilide | 11.3 (9.4-13.0) | 10.5 (6.3-14.9) |
| Dolasetron * | 9.9 (8.0-11.7) | 7.4 (3.0-11.0) |
| Moxifloxacin | 9.6 (7.7-11.4) | 14.5 (10.5-17.7) |
| Ondansetron | 16.6 (13.7-19.6) | 9.7 (6.2-12.8) |
| Quinine | 7.7 (4.5-10.8) | 11.6 (6.8-17.1) |
| Negative drug Levocetirizine | Not detected | 2.1 (-2.3-6.1) |

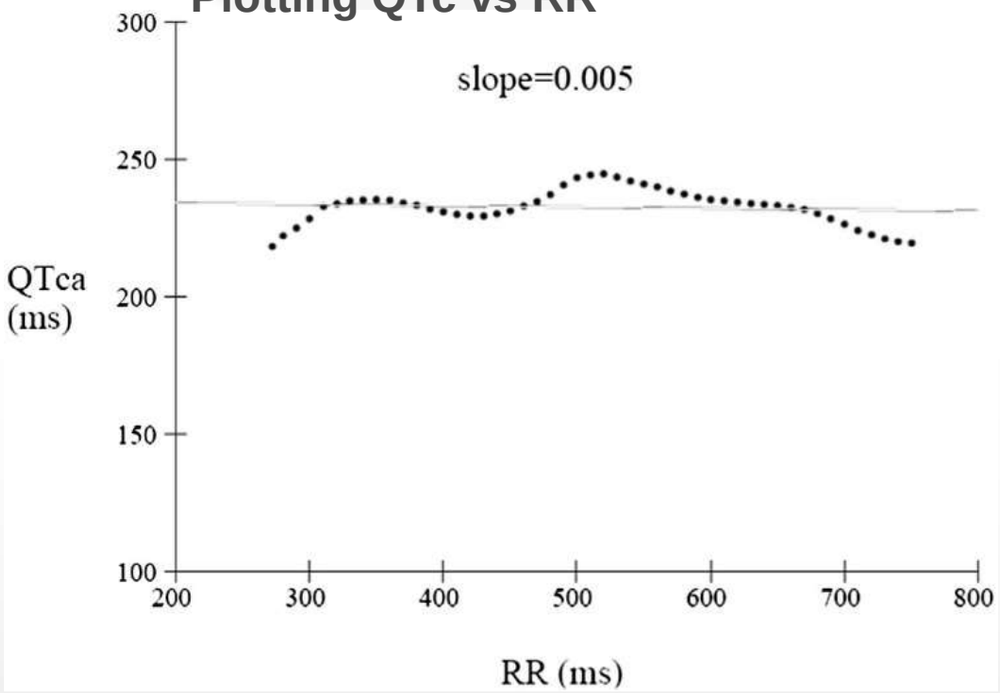
***:** Hydrodolasetron (main metabolite), (  ): Lower bound-upper bound of 90% CI
**#:** Parameters and predictions driven from a linear mixed-effects model in IQ-CSRC study (Darpo B *et al.*, 2015).

**Nonclinical E-R modeling could identify the QT effect consistently with the outcomes in humans**

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

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

**Plotting QTc vs RR**




**Additional information**

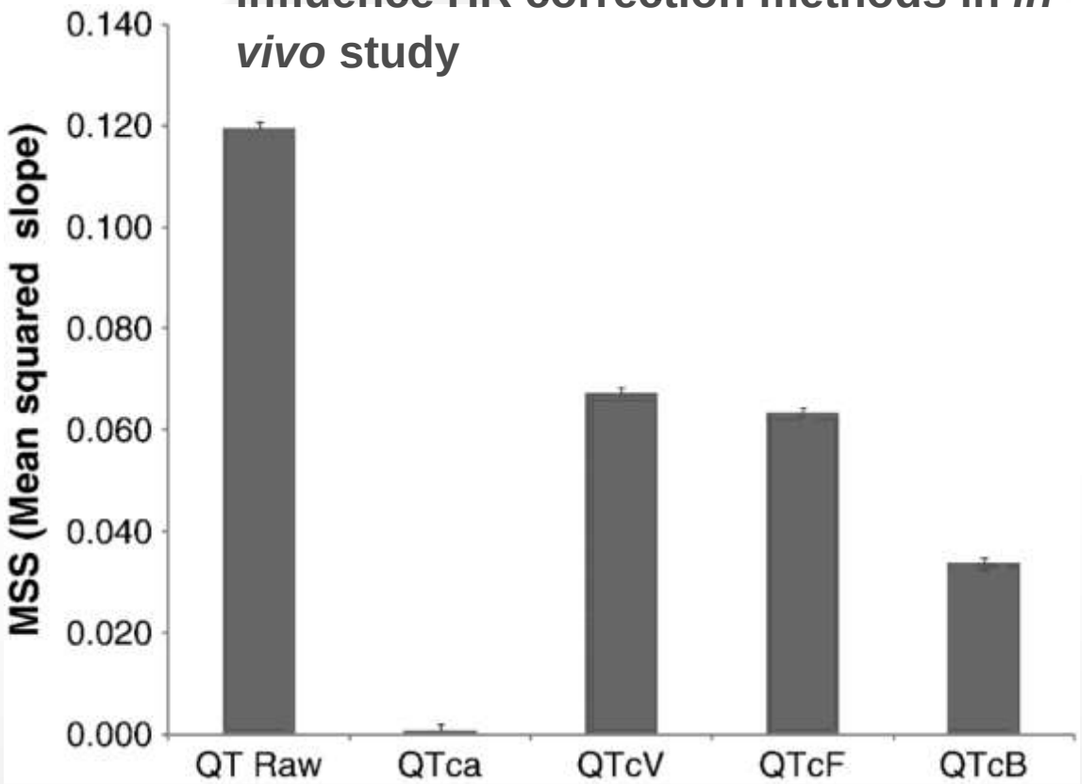
- Number of matched QTc-RR pairs
- Correlation metric
- 95% confidence interval
- P-values

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

**(Holzgrefe H. *et al.*, 2014)**

# Q&A 3.3: Individual QT Correction Based on QT-RR Relationship is Preferred With The Drug Affects Heart Rate

**Influence HR correction methods in *in vivo* study**




(Holzgrefe H. *et al*, 2014)

**Individual rate-corrected QT (QTca) is suitable, when a drug affects HR.**

**Example for 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)

**HR correction methods for human**

- QTcV: Van de Water
- QTcF: Fridericia
- QTcB: Bazett

# Q&A 3.4: Assessing Assay Sensitivity

- **The test system for an *in vivo* QT assay should provide a robust response**
- **As a positive control is not routinely used in the *in vivo* QT assay, assay sensitivity is commonly validated when introducing / changing test conditions (e.g., ECG system, animals) in each laboratory**
- **If study results will support ICH E14 Q&A 6.1, then the study should be powered to detect a QTc prolongation effect of a magnitude similar to dedicated clinical QT studies**
- **The best practice considerations provide guidance for assessing assay sensitivity, including when using historical positive control data**

# Q&A 3.4: How to Demonstrate Assay Sensitivity

- **Demonstration of assay sensitivity in each laboratory can be achieved by:**
  - ✓ **Defining minimum detectable differences (MDD) of positive controls and testing the effects of positive controls**

- **Statistical power calculations could also be provided from historical positive data from the same laboratory using an identical protocol**

- **If historical positive control data are utilized to justify assay sensitivity or if statistical power is calculated from historical control data:**
  - ✓ **Variance of the present data should be consistent with that seen historically**

# 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 percent change from baseline, confidence interval**
  - **P-value for changes from baseline and vehicle control**

- **PK content**

  - **Summary statistics for Cmax, AUC and Tmax for parent drug and metabolite (by table)**
  - **Time plot vs. plasma concentration for parent dung and metabolite (by figure)**

# Summary

**Best practices in Q&A 3.1 – 3.5 could be applied when conducting current *in vivo* QT assays**

- **Q&A 3.1, species selection and study design**
  - **Conscious freely-moving telemeterized non-rodent animals are customary**
- **Q&A 3.2, exposure assessment**
  - **E-R modeling may be helpful in the circumstances when QT prolongation is observed or anticipated based on hERG assay results**
- **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**
  - **Best practice for assay sensitivity could be applied when introducing new / changing test conditions (ECG system, animals etc.) in each laboratory.**
- **Q&A 3.5, presenting the PD and PK results**

# Summary

**Best practices in Q&A 3.2 and 3.4 should be applied *in vivo* QT assay;**

***when study results will support ICH E14 Q&A 5.1 and 6.1,***

- **Q&A 3.2, exposure assessment**
  - **E-R modeling can be helpful when the nonclinical *in vivo* QT study should be powered to detect an effect similar to dedicated QT studies in humans**
  - **The representative test conditions for E-R modeling are shown in Q & A 3.2.**

***when study results will support ICH E14 Q&A 6.1,***

- **Q&A 3.4, assay sensitivity**
  - ***In vivo* QT assay system in each laboratory could be justified assay sensitivity by using positive control or by utilizing historical positive data to define variance and sensitivity**

# References


- Darpo B., Benson C., Dota C., Ferber G., Garnett C., Green C ., Jarugula V., Johannesen L., Keirns J., Krudys K., Liu J., Ortemann-Renon C., Riley S., Sarapa N., Smith B., Stoltz R R., Zhou M., Stockbridge N. (2015). Results from the IQ-CSRC prospective study support replacement of the thorough QT study by QT assessment in the early clinical phase. Clinical Pharmacology & Therapeutics, 97, 326-335. https://ascpt.onlinelibrary.wiley.com/doi/abs/10.1002/cpt.60
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# Thank you!

**Satoshi Tsunoda**

**Best Practice Considerations for the *In vivo* QT Studies**

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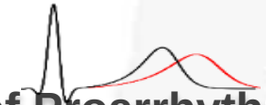
for Pharmaceuticals for Human Use

# Day 2 Schedule

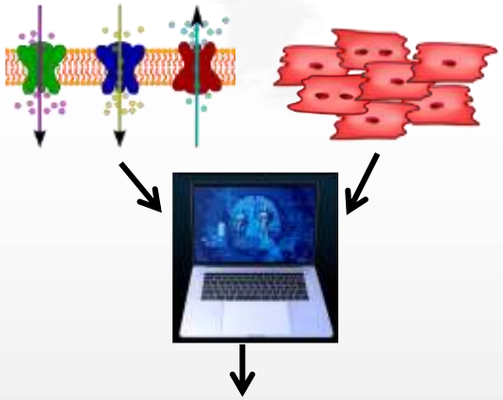
**Best Practice Considerations**

***In vitro* studies**

***In vivo studies***



**Principles of Proarrhythmia Models**




- ✓ **Recap of Day 1 and Introduction to Day 2**
  - ✓ **Derek Leishman, *PhRMA***
- ✓ **Best Practice Considerations for *In vitro* Studies Q&As**
  - ✓ **Wendy Wu, *FDA, United States* and Gary Gintant, *PhRMA***
- ✓ **Best Practice Considerations for *In vivo* QT Studies Q&As**
  - ✓ **Satoshi Tsunoda, *MHLW/PMDA, Japan***
- ➢ **Principles of Proarrhythmia Models Q&As**
  - **Takashi Yoshinaga, *JPMA***
- ➢ ***Discussion of Questions Received from the Q&A Pod***
  - **Facilitators: Derek Leishman, *PhRMA* and David Strauss, *FDA, United States***
  - **All Speakers and Xiaodong Zhang, *NMPA, China*; Eva Rached, *Swissmedic, Switzerland*; and Yu-Chung Chiao, *TFDA, Chinese Taipei; Katsuyoshi Chiba, JPMA***