企画：厚生労働省／独立行政法人医薬品医療機器総合機構／日本製薬工業協会

ICH E14/S7B Implementation Working Group：
「QT/QTc 間隔の延長と催不整脈作用の潜在的可能性に関する臨床的及び非臨床的評価」に関するQ&A説明会
（2023 年3 月16 日）

Best Practice Considerations for the In vivo QT Studies

In vivo QT試験に関するベストプラクティスの考慮事項について

Katsuyoshi Chiba, PhD
(Japan Pharmaceutical Manufacturers Association/Daiichi Sankyo Co., Ltd.)

千葉　克芳（日本製薬工業協会/第一三共株式会社）

JPMA

Best Practice Considerations for the 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?
  動物種選択及び一般的な試験デザイン
- **Question 3.2**: What should be considered for **exposure assessment** during the *in vivo* QT study?
  曝露評価
- **Question 3.3**: What information is needed to support the choice of **heart rate correction method** in an *in vivo* QT assay?
  心拍数によるQT補正方法
- **Question 3.4**: How should the sensitivity of the assay be evaluated?
  測定法の感度
- **Question 3.5**: What are the recommended conventions for **presenting the pharmacodynamic and pharmacokinetic results** of an *in vivo* QT assay?
  薬力学及び薬物動態の試験成績の提示内容

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**
  コアバッテリー試験として貢献
- **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**
  試験システム、実験条件、解析方法、報告様式やQTc感度が様々であることが判明
  （データの信頼性を低下させる要因）
- **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**
  教訓を生かし、意思決定のための価値を付加し、信頼性を高めるための考慮事項を示す必要
- **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**

  非臨床試験は統合的リスク評価へ寄与できる（E14 Q&A 5.1／6.1に使用される場合）

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

最も最適な非げっ歯類の選択及びその正当性

- **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)**

  一般毒性試験と同じ動物種（非げっ歯類）を使用

- **Conscious freely-moving telemeterized animals are customary**

  覚醒かつ自由行動下のテレメトリー機器を装着した動物

- **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**

  代替モデルが有用になる場合（十分な曝露の担保や化合物特有の問題点に対応する目的）

Q&A 3.2: Considerations for Achieving Adequate Drug Exposure

- **S7B states that drug exposures should include and exceed anticipated therapeutic concentrations**
  予想される治療濃度を含み、かつそれを超える濃度の設定

- **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**
  予想される高い臨床曝露量を網羅（E14 Q&A 5.1／6.1に使用される場合）

  - **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**
    高い臨床曝露量は、定常状態における平均最高血中濃度の上昇に大きな影響を及ぼす内因性または外因性要因の存在下で、最大治療用量の投与時の曝露量と定義
  - **As noted in ICH S7B, the dose range can be limited by animal intolerance to the test substance**
    用量範囲は被験物質に対する動物の不忍容性により限定される場合がある

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**
  QT評価に用いた同じ動物での曝露評価が推奨
  - **Exposure data from a separate PK and toxicity study can be used**
    別のPK試験や一般毒性試験の成績を用いることも可能

- **Blood samples should be taken at relevant time-points and in a manner that limits interference with QT assessment**
  適切な時点におけるQT評価の妨げにならない血液サンプリング
  - **Can be done by sampling complete PK profiles in the same animals on a separate day after an adequate washout**
    別の日に同じ動物で詳細なサンプリング
  - **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**
    QT評価時に少なくとも1時点のサンプリング

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**
  十分なPKサンプリングを行うことにより、臨床QT試験の薬物濃度 - QTc解析と類似したERモデリングが可能

- **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.
  ヒトQT試験と同程度の作用の検出力が求められる場合に有用となり得る

- **In addition, exposure-response modeling may be helpful in other circumstances when QT prolongation is observed or anticipated based on hERG assay results**
  hERG試験成績に基づきQT延長が認められる又は予想される場合にも有用となり得る

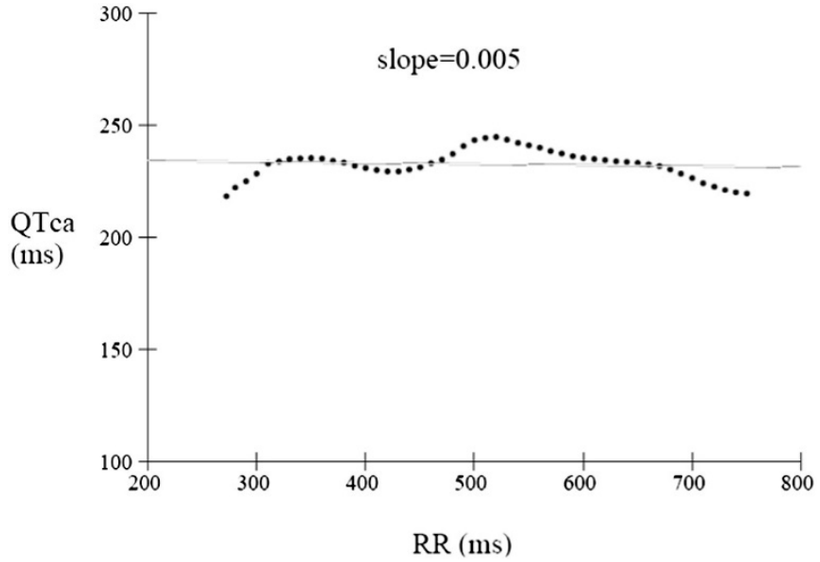
- **Representative references for nonclinical in vivo concentration-QTc modeling**
  - **Dubois et al. British Journal of Clinical Pharmacology 2017 (Dog)**
  - **Komatsu et al. Journal of Pharmacological and Toxicological Methods 2019 (Monkey)**
  - **Chui et al. Clinical and Translational Science 2021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

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

QTc間隔とRR間隔を対比するプロット及び付随するその他の情報（マッチさせたQTc－RRペア数、相関指標、95%信頼区間、p値など）により、QTc間隔とRR間隔との間に相関性がないこと（心拍数の影響を受けないこと）を示す

Example plot demonstrating independence of QTc vs. RR



QTca: Individual rate-corrected QT

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)**

被験物質が心拍数に影響を及ぼす場合には、一般的な方法（Bazett、Fridericia、Van de Waterなど）と比較して、精度及び感度が高いとされるQT-RR関係に基づく個別のQT補正が望ましい

Q&A 3.4: Assessing Assay Sensitivity – General Recommendations

- **The test system should provide a robust response** ロバストな反応が得られること
- **Assay sensitivity of relevant functional endpoints should be evaluated and reported to enable data interpretation and contextualization**
  アッセイ感度を評価し、それに基づきデータを解釈し、報告書を作成する

    - **Demonstration of assay sensitivity can be achieved by defining minimum detectable differences (MDD) and testing the effects of positive controls**
      アッセイ感度は、検出可能な最小差（MDD）の定義及び陽性対照を用いた試験によって説明可能

    - **Statistical power calculations could also be provided from historical data from the same laboratory using the identical protocol**
      統計学的な検出力は、同一の試験方法を用いた同一の検査施設における背景データを用いて算出可能

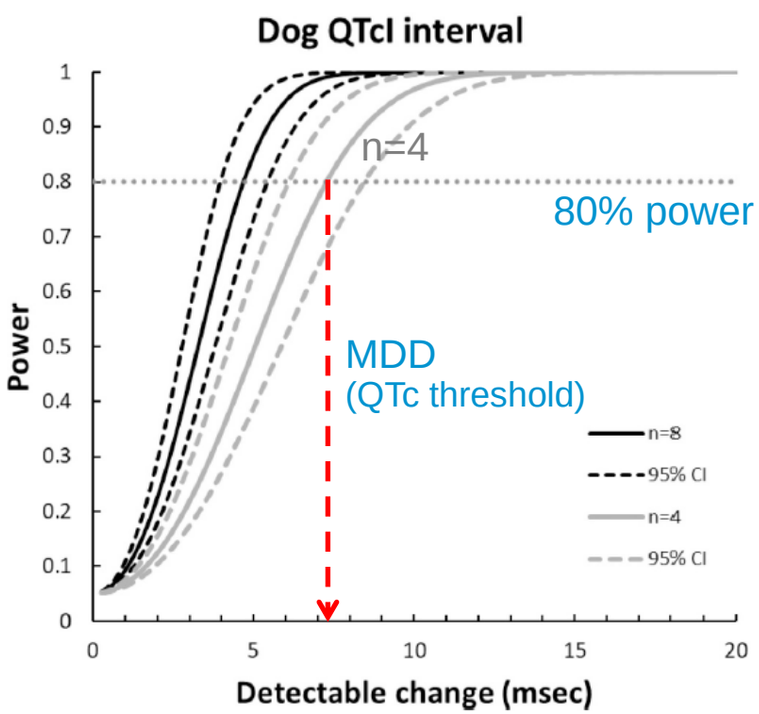
        - If historical positive control data are utilized to justify assay sensitivity or statistical power is calculated from historical control data, then the variance of the present data should be consistent with that seen historically
          背景データを用いてアッセイ感度の正当性を立証する場合又は統計学的な検出力を算出する場合、試験成績の分散が背景データの分散と一致することを示す

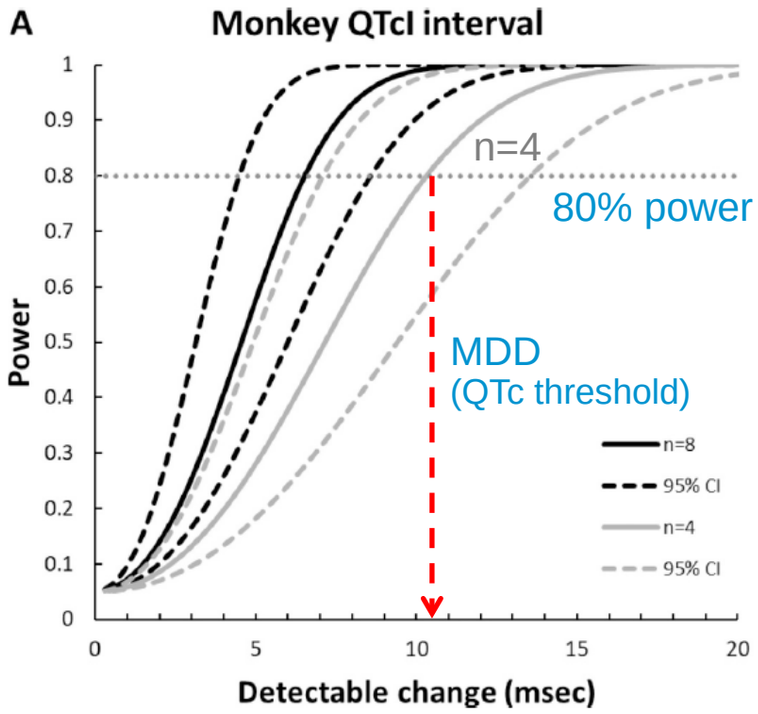
Minimum Detectable Difference (MDD)

- **The 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

- MDDは最小の効果サイズ
- レトロスペクティブな検出力分析により決定
- 感度と再現性を追跡するために使用

n=4



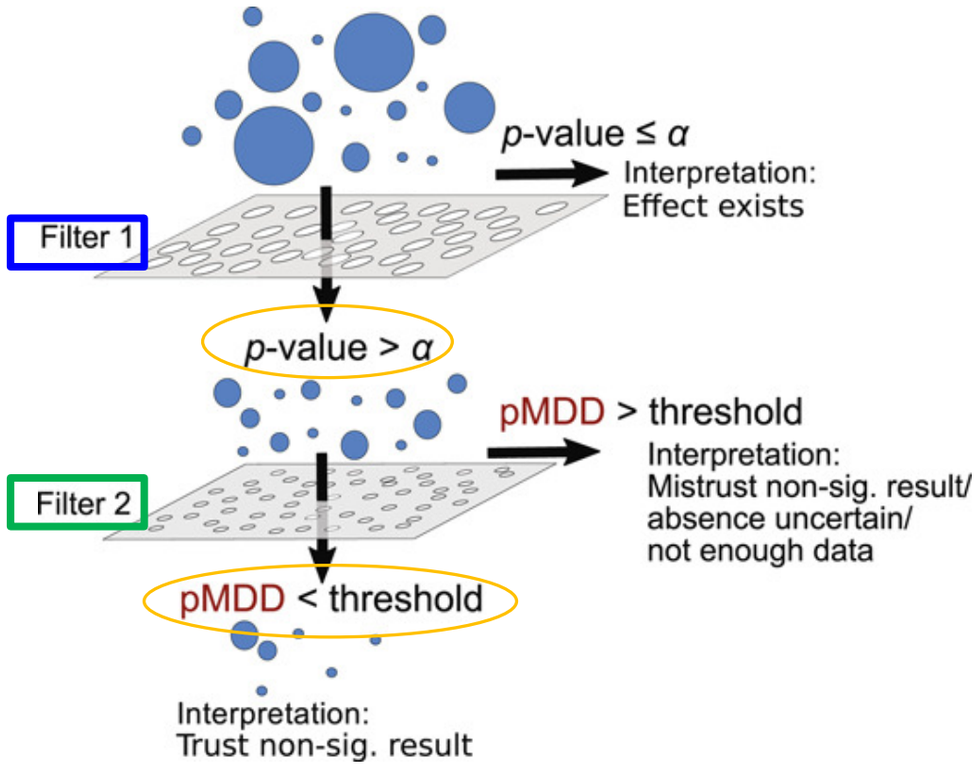


- MDD is calculated from residual error, sample size, significant level, and power
- True treatment difference that will be claimed statistically significant with a high probability (e.g., 80%, p<0.05)

- MDDは、動物種や試験デザインによって異なるが、小さなQTc効果（e.g. 10 msec）を検出可能

Baublits J et al. Journal of Pharmacological and Toxicological Methods 2021

Minimum Detectable Difference (MDD)



Mair MM et al. Environmental Toxicology and Chemistry 2020

Filter 1: Significance test (e.g., ANOVA)

Large effects detected (p ≤ α)

Small effects undetected (p > α) （有意ではない）

Filter 2: MDD

When MDD < QTc threshold, “no effect on QTc” can be trusted

（MDD < QTc閾値の場合、QTcに影響なし）

- Each experimental endpoint needs to pass to be considered showing “no effect”
- MDD confirms that the study power is sufficient to trust nonsignificant or no-effect findings (i.e., filter 1 results)

陰性であることをMDDにより補完することが可能

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**
  試験毎に陽性対照の設定は不要であり(3Rs原則に従う)、アッセイ感度は各試験施設において試験系の導入や変更した場合に検証

- **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**
  臨床QT試験と同程度の検出感度が認められることが必要(E14 Q&A 6.1に使用される場合)

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**
  感度は、心電図評価及び高い臨床曝露量と比較した*in vivo*試験で得られた曝露量の両方に依存

- **Hypothetical example presented in the Q&A:**
  - The 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

  仮説的な例：最小検出差は、高い臨床曝露量のみを網羅している場合は5 msec。高い臨床曝露量のより大きな倍数が達成された場合はそれより高くなる（例えば、3倍高い臨床曝露量が達成された場合は10 msec、さらに大きい倍数が達成された場合は、QTc閾値がさらに高くなる）

- **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

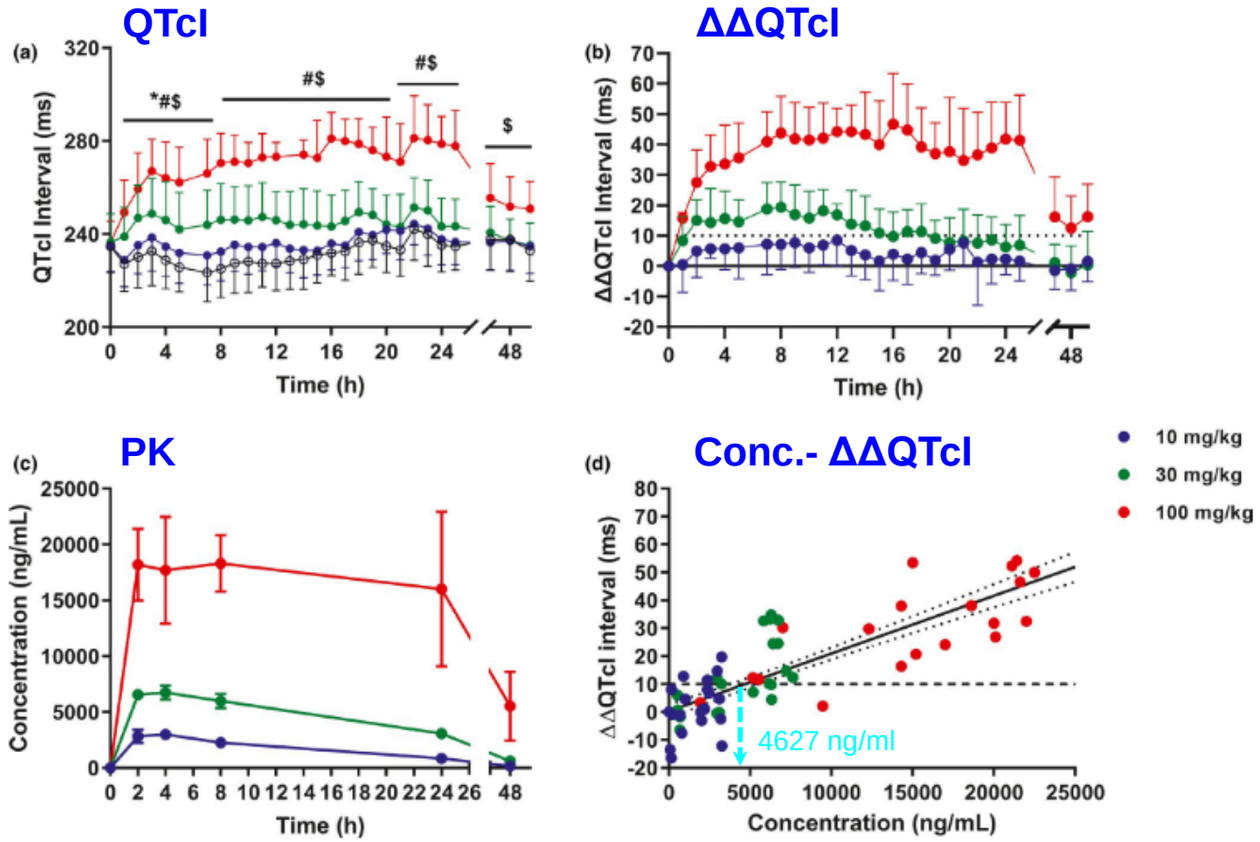


Figure 1 Time-response and concentration-QTc (C-QTc) relationship evaluation of moxifloxacin-induced QTc prolongation in conscious beagle dogs. Vehicle (o) and moxifloxacin (10, 30, and 100 mg/kg) were administered at 0 h. The plots represent timepoint analysis of absolute QTcI (a) and baseline- and 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.

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

***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.**

10 msecのQTc変化を生じるモキシフロキサシンのフリー濃度は、ヒトTQT試験のそれより2-2.5倍大きい

Training Materials Examples for ICH E14 Q&A 6.1

Table 2-D. In Vivo QT Assessment

QT Study

Exposure	The 30 mg/kg dose provides a 5.0-fold margin over high clinical exposure scenario
Design[1]	*Crossover, N=4*
Species:	*Dogs*
Historical Sensitivity:	*MDD: 8 ms (95% CI: 6, 10)*
	Sensitivity at critical concentration for moxifloxacin: 3.6 ms
ECG collection	*24-h telemetry*
ECG reading methodology	*Fully automated*
PK Collection	*Same study, at 3 h post-dose*
	Cmax characterized at same dose levels in Toxicokinetic Study
Analysis Methods:	
Data reduction method	*0-3 h, 3-8 h, 8-12 h, 12-18 h, 20-24h after dosing (super-intervals)*
Analysis methodology	*By-time window using ANOVA*
HR correction method	*QTcI based on 24 h baseline data in each animal*
ECG Findings	*No ventricular tachyarrhythmias*

Summary Findings

Moiety & Dose	*QTcI Effect Size (ms ± SE)*[2]	*Parent concentration at 3 h (ng/mL)*[3]	*C_{max}-total (ng/mL)*[4]	*C_{max}-free (ng/mL)*[5]	*Protein Binding: Species (%)*[6]	*High Clinical $C_{max,ss}$ (ng/mL)*[7]	*Exposure Ratio*[8]
	No QT effect						
3 mg/kg	0 ± 4	55	60	59	1% (dog) 1% (human)	*291 (95% CI: 265, 319)*	0.2
10 mg/kg	2 ± 5	595	582	576			2.0
30 mg/kg	4 ± 3	1550	1455	1440			5.0
MDD	10 ms						
Historical Positive Control Effect (Moxifloxacin)							
10 mg/kg	5.9 ± 1.3	ND	2980	2116	29 (dog) 40 (human)	Critical Concentration: 1866 ng/mL (free: 1120)	1.9
30 mg/kg	17.4 ± 2.8	ND	6730	4778			4.3
100 mg/kg	45.5 ± 3.7	ND	18300	12993			11.6

- MDD（10 ms）は、背景データのMDD［8 ms (95% CI: 6,10)］と同等（MDD幅の上限）
- モキシフロキサシンの濃度-QTc解析により、フリー濃度（1120 ng/mL）でのQTc間隔の延長は3.6 msec
- 曝露量が高い臨床曝露量のみを網羅している場合には約1/3（3.6/10）の感度であり、観察されたMDDに基づく臨床QT試験と同程度の感度を持たせるためには、曝露量比が少なくとも3倍必要
- 高い臨床曝露量の5倍の用量（30 mg/kg）でQTc間隔の延長はなし（MDDより低値）
- 臨床QT試験において、モキシフロキサシンのフリー濃度（1120 ng/mL）でのQTc間隔の延長は10 msec

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

規制当局による審査を容易にするための一般的な推奨事項

Pharmacodynamic (PD) content: 薬力学パラメータ

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 MDD with by time analysis 時間分析による検出可能な最小差
- providing that the data for the new drug and the historical data were collected according to the same protocol and statistical analysis plan 背景データの提示、データの分散の一致を示す
- If deviations are present, they should be clearly justified 逸脱がある場合はその正当性を示す

If concentration-QTc modeling is performed;

- Reporting should follow similar principles as for human concentration-QTc modeling (see ICH E14 Q&A 5.1) ヒト濃度 - QTcモデリングと同様の原則に従う

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

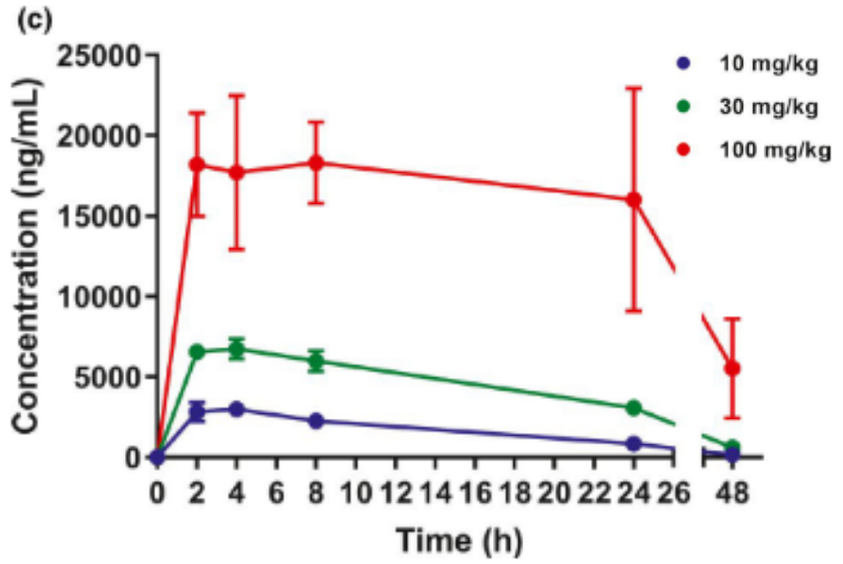
Pharmacokinetic (PK) content: 薬物動態パラメータ

- **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 (by Table).**
- **Time plot vs. plasma concentration for parent drug and metabolite (by Figure)**

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)

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** 時点毎の統計解析
  - Assay sensitivity is highly dependent on the experimental design and statistical methodology utilized
    アッセイ感度は試験デザインや統計手法に依存
  - A sensitive statistical methodology, such as analysis of variance (ANOVA), is recommended for study designs that assess treatment, animal and period effects
    変動要因である薬物処理、動物、及び実験日の効果を評価する試験デザインにおける統計手法の推奨（ANOVA）
    - ANOVA can be applied to both cross-over designs and parallel group designs
      ANOVAはクロスオーバー及びパラレルデザインの両方に適用可
    - Representative references: Aylott et al. (2011), Derakhchan K et al. (2014), Chui R et al., (2021)

- **Examples of Statistical Analysis:** 統計解析例
  - **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
  - **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

3R（動物数の削減／苦痛の軽減／代替法の利用）原則に従って動物の使用数を減らすこと

- **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 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

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- **EC, Europe**
  - Dr. Frank Holtkamp
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- **EFPIA**
  - Dr. Charles Benson
  - Dr. Corina Dota
  - Dr. Jean-Pierre Valentin
- **FDA, United States**
  - Dr. David Strauss
  - Dr. Christine Garnett
  - Dr. John Koerner
  - Dr. Wendy Wu
  - Dr. Zhihua Li
- **Health Canada, Canada**
  - Dr. Colette Strnadova
- **JPMA**
  - Dr. Katsuyoshi Chiba
  - Dr. Maki Ito
  - Dr. Takashi Yoshinaga
- **MHLW/PMDA, Japan**
  - Dr. Satoshi Hoshide
  - Dr. Wataru Kuga
  - Dr. Satoshi Tsunoda
  - Dr. Kaori Shinagawa
  - Dr. Jun Oikawa
- **NMPA, China**
  - Dr. Xiaodong Zhang
  - Dr. Shuiqiang Wang
- **PhRMA**
  - Dr. Gary Gintant
  - Dr. Derek Leishman
  - Dr. Hugo Vargas
- **Swissmedic, Switzerland**
  - Dr. Eva Rached
  - Dr. Thomas Kleppisch
- **TFDA, Chinese Taipei**
  - Dr. Yu-Chung Chiao