# Utility of Nonclinical Cardiac Repolarization Assays to Predict Clinical $QT_C$ Outcomes for Peptide and Protein Therapeutics

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## Abstract

The current cardiac safety testing paradigm centers on detecting drug-induced repolarization delay as a surrogate endpoint for the rare but potentially fatal ventricular arrhythmia Torsades de Pointes. The nonclinical strategy described in the ICH S7B guideline includes *in vitro* testing of drug block of hERG channels that repolarize ventricular myocytes, and *in vivo* QT assessment using non-rodent species. Several studies have found this nonclinical strategy to be adequate for identifying small molecules with clinical $QT_C$ liability, thereby supporting the use of hERG and *in vivo* QT data to inform the design of first-in-human studies. In contrast, monoclonal antibodies (mAbs) are large targeted proteins that exhibit low $QT_C$ prolongation risk. The nonclinical testing strategies for mAbs are described in ICH S6, which does not recommend the hERG assay, and indicates that cardiovascular endpoints may come from toxicology studies. In between small molecule drugs and mAbs are intermediate-sized molecules including peptides and proteins. There are two questions for these molecules: 1) Are they associated with clinical $QT_C$ liability? 2), If so, are hERG and *in vivo* QT data useful to predict this liability? To address these questions, a database for peptide and protein products (excluding mAbs) submitted to the FDA for marketing approval was generated, and results for the *in vitro* hERG assays, *in vivo* QT assessments, clinical QT studies, and cardiac effects stated on the product labels (if approved) were collected. This study found that 19% of approved peptides and proteins have $QT_C$ prolongation language on the label. However, most are similar products for similar indications. Clinical $QT_C$ results for approved and investigational products combined show that 12.5% were positive. HERG assay lacks sensitivity, while *in vivo* QT assessment exhibits sensitivity and positive predictive power for clinical $QT_C$ prolongation. Thus, the hERG assay is unsuitable for assessing the mechanism of $QT_C$ prolongation for peptides and proteins. ICH S7B and E14 guidelines are now open for modification through Q&A mechanism. This study provides information that can be considered by the ICH working group updating these two regulatory guidelines.

## Introduction

- Two ICH guidelines were implemented in 2005 to prevent unanticipated drug induced QTc prolongation and Torsade de Pointes:
  - S7B: *in vitro* and *in vivo* QT studies to assessing the potential of a test substance to delay ventricular repolarization
  - E14: Clinical studies to characterize QT/QTc interval changes for drugs with systemic bioavailability
- Prior studies have demonstrated an association between hERG block[1-4] or in vivo QT prolongation[1,3-5] and clinical QT prolongation, but predominantly focused on small molecules (<1 kDa).
- Many small molecules block hERG channels by binding to common amino acid residues with side chains projecting toward the $K^+$ permeation pathway, accessed from the intracellular side of the membrane.[6,7]

## Introduction

- Large targeted molecules, specifically, monoclonal antibodies (mAbs) that are long amino acid polymers (~50 to 155 kDa for FDA-approved ones), do not cross cell membranes easily and have been found to have a low likelihood of directly interacting with the hERG channel.[8] Clinical QT studies are therefore not recommended for such products unless there is a mechanistic basis for concerns.
- Between the small molecules and large mAbs are a diverse group of intermediate sized molecules including peptides and proteins. The size range of this group is quite broad, and ranges from 0.44 kDa (lisinopril) to abobotulinumtoxinA (147.3 kDa).
- The risk of QTc prolongation and utility of preclinical assays to predict clinical QTc prolongation for these intermediate sized molecules is unclear.

## Methods

***Database***

- A total of 242 unique peptides and proteins were submitted to the FDA between March 1952 to September 2020. Fc and PEG products were included in this database; mAB, excluded.
- Forty-six products had hERG results; 66, *in vivo* QT; and 53, clinical QT studies.

***Data evaluation of clinical and nonclinical studies***

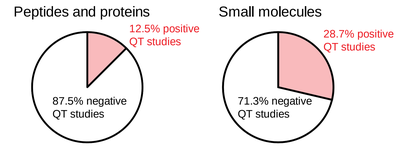
- Clinical QT studies were analyzed by the FDA IRT per definition in ICH E14.
- Concentrations tested in nonclinical studies were compared with free or total $C_{max}$ after administering therapeutic or maximum dose in humans.

***Statistical analysis***

- Performance of hERG or *in vivo* QT assay in correctly identifying clinical $QT_C$ outcomes was assessed by generating sensitivity, specificity, and other related measures.
- Sensitivity (= TP / (TP + FN)) reflects the proportion of positive nonclinical studies that correctly identifies products associated with clinical $QT_C$ prolongation.
- Specificity (= TN / (TN + FP)) reflects the proportion of negative nonclinical studies that correctly identifies products that did not prolong the $QT_C$ interval in clinical studies.
- Positive and negative predictive values (PPV and NPV, respectively) were also calculated using the following equations to yield the probability of each nonclinical study outcome in correctly predicting the positive or negative clinical outcome: PPV = TP / (TP + FP); NPV = TN / (TN + FN).

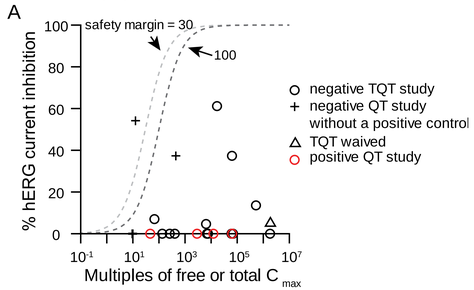
## Results and Discussion

**Figure 1. Percent of positive clinical QT studies for peptides and proteins is lower than that for small molecules.**




In this database, 5 out of 40 clinical studies were positive. Another FDA database by Park et al., 2018[3], comprised of 95% small molecules, showed that 43 out of 150 clinical studies were positive.

**Figure 2. *In vitro* hERG assay lacks sensitivity for clinical $QT_C$ prolongation for peptides and proteins.**



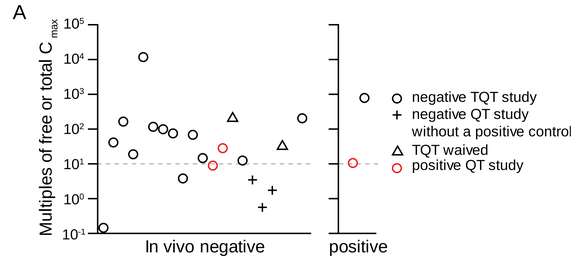

B

in vitro hERG results

| Clinical QT results | Predicted positive | Predicted negative | | |
|---|---|---|---|---|
| n = 21 | | | | |
| Actual positive | TP = 0 | FN = 4 | Sensitivity | 0 |
| Actual negative | FP = 1 | TN = 16 | Specificity | 0.94 |
| | | | PPV | 0 |
| | | | NPV | 0.80 |

Twenty-one products had both hERG and clinical QT results. These products ranged from 3 to 550 amino acids in length (0.53 to 59.7 kDa), hence included both peptides and proteins. **A)** HERG current inhibition vs. highest *in vitro* concentration tested, expressed as multiples of free or total $C_{max}$. The light and dark dotted gray curves represent hypothetical products with safety margins of 30 and 100, respectively. The product to the left of the dotted gray curves was considered hERG positive; the rest, hERG negative. **B)** *Left panel*, confusion matrix summarizing outcomes of the hERG assay (predicted results) relative to clinical $QT_C$ outcomes (actual results). Right panel, performance measurements.

**Figure 3. Performance of *in vivo* QT assessment in aligning and predicting clinical $QT_C$ prolongation.**




B

All products with in vivo & clinical QT results

In vivo QT results

| Clinical QT results | Predicted positive | Predicted negative | | |
|---|---|---|---|---|
| n = 22 | | | | |
| Actual positive | TP = 1 | FN = 2 | Sensitivity | 0.33 |
| Actual negative | FP = 1 | TN = 18 | Specificity | 0.95 |
| | | | PPV | 0.50 |
| | | | NPV | 0.90 |

C

Products with ≥10X free or total $C_{max}$

In vivo QT results

| Clinical QT results | Predicted positive | Predicted negative | | |
|---|---|---|---|---|
| n = 16 | | | | |
| Actual positive | TP = 1 | FN = 1 | Sensitivity | 0.50 |
| Actual negative | FP = 1 | TN = 13 | Specificity | 0.93 |
| | | | PPV | 0.50 |
| | | | NPV | 0.93 |

Twenty-two products had both *in vivo* and clinical QT results. These products range from 3 to 550 amino acids in length (0.53 to 59.7 kDa), hence included both peptides and proteins. **A)** The highest dose tested in *in vivo* QT studies, expressed as multiples of free or total $C_{max}$ from human studies, for studies with negative and positive *in vivo* QT outcomes. Several retrospective studies had reported that the predictive performance of *in vivo* QT studies for clinical $QT_C$ prolongation was good at exposure levels 10X to 30X higher than clinical $C_{max}$. A dotted gray line thus marks 10X free or total $C_{max}$, **B, C)** *Left panel*, confusion matrix summarizing outcomes of the *in vivo* QT/$QT_C$ outcomes (predicted results) relative to clinical $QT_C$ outcomes (actual results) for all products (B), or for products with *in vivo* exposure level ≥10X multiples of free or total $C_{max}$. Right panel, various performance measurements for this assay.

## References

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## Conclusion

- Peptides and proteins have lower $QT_C$ prolongation risk than small molecules.
- Peptides and proteins that do prolong the $QT_C$ interval do not directly block hERG channels.
- These data can be considered by the international Council for Harmonization working group updating the ICH S7B and E14 regulatory guidelines. The concept paper from this working group shows agenda to set criteria for defining low risk molecules that may not need dedicated QT-focused evaluation.