Establishing the Internal hERG Safety Margin Threshold Using a Series of Reference Drugs to Support the Integrated QTc Risk Assessment

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Background

As ICH E14/S7B Q&As was adopted in February 2022[1], the pharmaceutical industry paid more attention to best practice considerations and hERG safety margin threshold. ICH E14/S7B Q&As require to use a hERG safety margin threshold to define negative hERG assays. Given that inter-laboratory variability in hERG IC50 persists even after implementing standardized protocols and adhering to ICH S7B Q&A best practices, it is wiser for each laboratory to establish its own hERG safety margin threshold rather than relying on a published one.

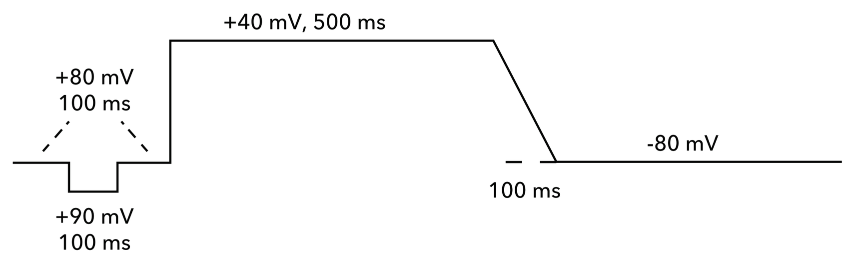
Method

Cell line: hERG-transfected CHO cells

Reference drugs: Cisapride, Dofetilide, Moxifloxacin, and Ondansetron

Electrophysiology:

- Voltage protocol: FDA recommended voltage protocol command (stimulation frequency: 0.2 Hz)


+40 mV, 500 ms
+80 mV
100 ms
-80 mV
100 ms
+90 mV
100 ms

Voltage protocol for hERG testing

- Recording temperature: near physiological temperature (35–37°C)
- Platforms: two laboratories both using manual patch-clamp system

Full blocker: 1 μM E-4031

Statistical analysis methodology[2,5]

- The analyses utilized 13 hERG IC50 values for the four selected reference drugs. Data from across drugs, laboratories, and repeated experiments were pooled using a random-effect meta analysis using the metafor package in R software.
- Log-normal distributions were assumed for both plasma concentration and hERG IC50.
- Using the mean and standard deviation to create large random data distributions.
- The margin is calculated by

$$\text{Margin} = \frac{\text{hERG } IC_{50}}{\text{Concentration} \times \text{Unbound Fraction}}$$

where Unbound Fraction=1-Plasma Protein Binding Fraction.

References

1. ICH E14/S7B Questions and Answers: Clinical and Nonclinical Evaluation of QT/QTc Interval Prolongation and Proarrhythmic Potential, 21 Feb 2022.
2. Leishman, Derek J et al. "Supporting an integrated QTc risk assessment using the hERG margin distributions for three positive control agents derived from multiple laboratories and on multiple occasions."
3. Pfeiffer, Emily R et al. "Specific prediction of clinical QT prolongation by kinetic image cytometry in human stem cell derived cardiomyocytes."
4. Meuldermans, W et al. "Excretion and biotransformation of cisapride in dogs and humans after oral administration."
5. ICH E14/S7B Q&As Training Material Examples Supplemental File.

Results

The four reference drugs were conducted for 2 to 5 times (Cisapride: 2; Dofetilide: 3; Moxifloxacin: 5; and Ondansetron: 3) in accordance with best practices in two laboratories. The typical current trace for the four reference drugs were presented in Figure 1, reflected the significant dose-dependent response. The inhibition of selective blocker (E-4031)was sufficiently high, that can indirectly reflect cell quality. The concentration-response curves for the four reference drugs were presented in Figure 2. The IC50 value and hill coefficient was reported as mean ±95% confidence interval to evaluate the uncertainty estimation. hERG safety margin threshold defined by reference drugs were presented in Table 1, including the critical concentration, Protein Binding, molecular weight, IC_{50} Distribution; shown as shown as 50th (2.5th, 97.5th) percentile.

Figure 1 Typical hERG current trace for the four reference drugs. A to D represents the following drugs: A for Cisapride, B for Dofetilide, C for Moxifloxacin, and D for Ondansetron

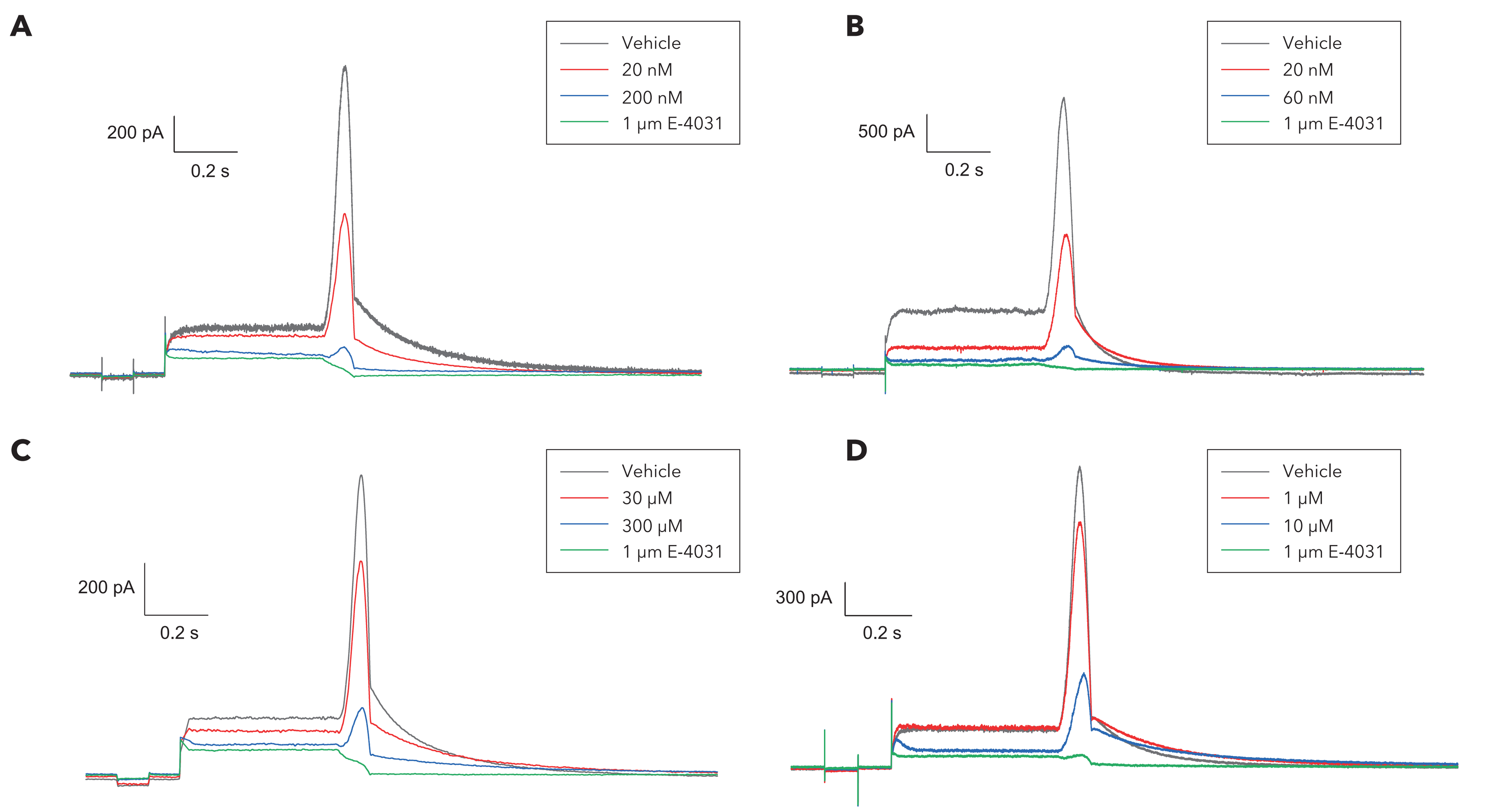
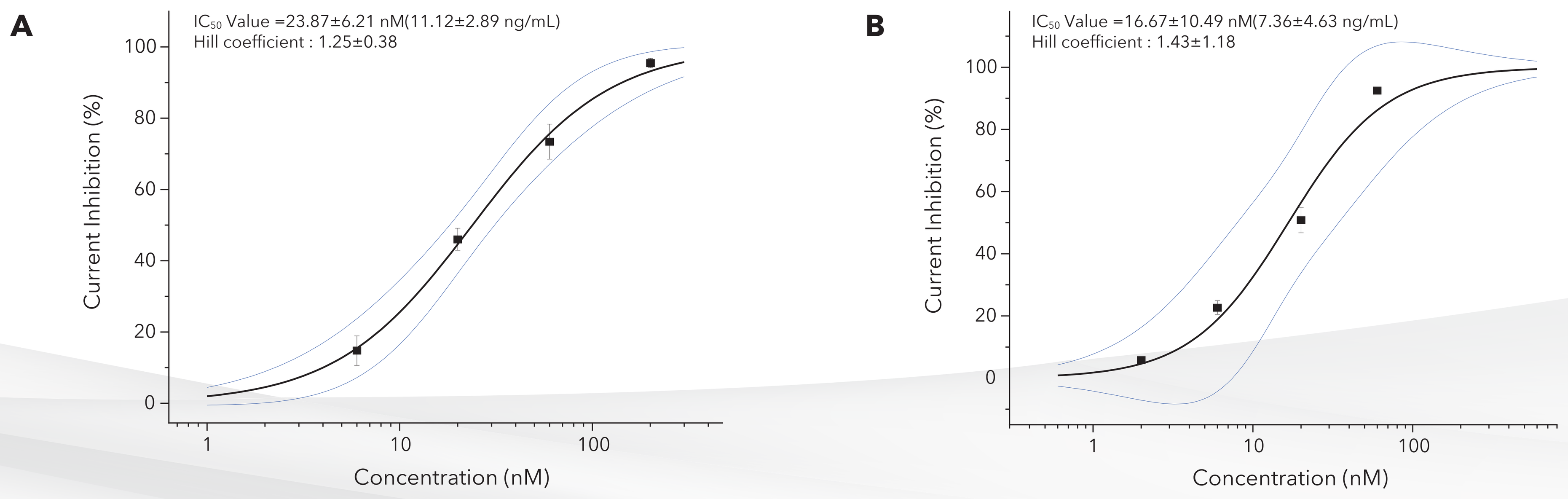

A
Vehicle
20 nM
200 nM
1 μm E-4031
200 pA
0.2 s
B
Vehicle
20 nM
60 nM
1 μm E-4031
500 pA
0.2 s
C
Vehicle
30 μM
300 μM
1 μm E-4031
200 pA
0.2 s
D
Vehicle
1 μM
10 μM
1 μm E-4031
300 pA
0.2 s

Figure 2 Concentration-response relationships for the four reference drugs. A to D represents the following drugs: A for Cisapride, B for Dofetilide, C for Moxifloxacin, and D for Ondansetron.


A
IC50 Value =23.87±6.21 nM(11.12±2.89 ng/mL)
Hill coefficient : 1.25±0.38
Current Inhibition (%)
Concentration (nM)
B
IC50 Value =16.67±10.49 nM(7.36±4.63 ng/mL)
Hill coefficient : 1.43±1.18
Current Inhibition (%)
Concentration (nM)

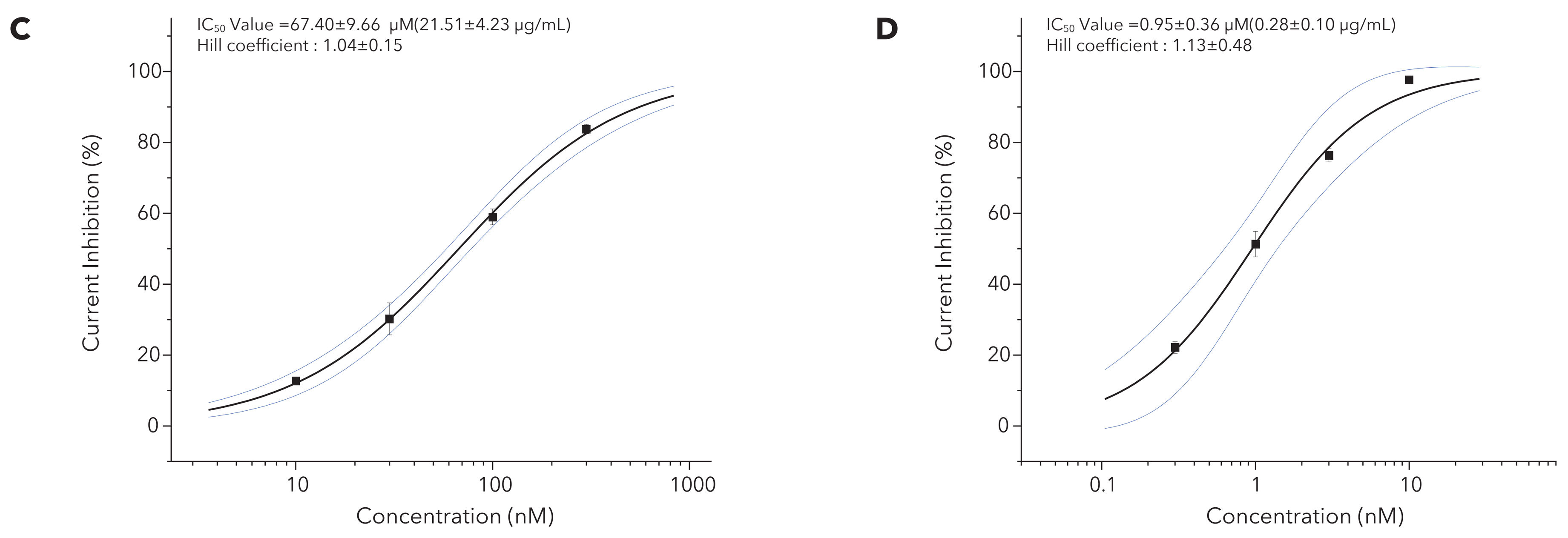

C
IC50 Value =67.40±9.66 μM(21.51±4.23 μg/mL)
Hill coefficient : 1.04±0.15
Current Inhibition (%)
Concentration (nM)
D
IC50 Value =0.95±0.36 μM(0.28±0.10 μg/mL)
Hill coefficient : 1.13±0.48
Current Inhibition (%)
Concentration (nM)

Table 1 hERG safety margin threshold defined by reference drugs

Reference Drugs	Critical Concentration (ng/mL)	Protein Binding, %	Mol Wt (g/mol)	IC_{50} Distribution (μM)	Safety Margin
Cisapride	135 (117, 155)[3]	97.5 (97.1, 97.9)[4]	466	0.02659 (0.02395, 0.02951) N=2	3.681 (2.910, 4.656)
Dofetilide	0.37 (0.24, 0.55)	64 (62, 66)	442	0.02562 (0.02383, 0.02756) N=3	84.79 (55.43, 129.7)
Moxifloxacin	1866 (1591, 2188)	40 (37, 43)	401	62.40 (55.56, 70.0958) N=5	22.31 (18.23, 27.31)
Ondansetron	249 (152, 412)	73 (71,76)	293	1.312 (0.9743, 1.767) N=3	5.696 (3.171, 10.23)
Pooled Safety Margin for Reference Drugs					14.11 (3.507, 56.78)
Threshold					>56.78

The validated outcomes, including simulated denominator exposure distributions and estimated margin distributions, were consistent with the ICH E14/S7B example, demonstrating the reliability of the methodology.

The mean and 95% confidence interval of the pooled hERG safety margin, as defined by reference drugs in our laboratories, are 14.11 (3.507, 56.78).

Conclusion

The IC_{50} values and variability were estimated using the best practice hERG protocol adhering ICH E14/S7B Q&As.

The threshold of approximately 57 (the upper bond of 95% confidence interval) in our laboratories is approaching the threshold of 51 proposed by the ICH S7B Q&A example, suggesting that a ~50-fold margin could offer sufficient discriminatory performance in an integrated risk assessment to identify drugs with low or no QTc liability.

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