THE ION CHANNEL EXPERT

How ChanTest Does CiPA: a Paradigm Shift for Cardiac Safety

4th ChanTest User Meeting May 9, 2014

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Adjunct Professor Physiology & Biophysics, CWRU School of Medicine
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Overview

• Present paradigm: S7B & E14 Guidances

• Problems

• Future guidance: the Comprehensive *in vitro* Proarrhythmia Assay (CiPA)
## Non-Cardiac Drugs and the hERG/ TdP “Epidemic”

It’s all about HERG!!

<table>
<thead>
<tr>
<th>Drug</th>
<th>Class</th>
<th>Date Withdrawn</th>
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</thead>
<tbody>
<tr>
<td>Terodiline</td>
<td>Uninary Incontinence</td>
<td>1991</td>
</tr>
<tr>
<td>Sparfloxacin</td>
<td>Antibiotic</td>
<td>1996</td>
</tr>
<tr>
<td>Terfenadine</td>
<td>Antihistamine</td>
<td>1998</td>
</tr>
<tr>
<td>Astemizole</td>
<td>Antihistamine</td>
<td>1999</td>
</tr>
<tr>
<td>Grepafloxacin</td>
<td>Antibiotic</td>
<td>1999</td>
</tr>
<tr>
<td>Cisapride</td>
<td>Prokinetic</td>
<td>2000</td>
</tr>
<tr>
<td>Droperidol</td>
<td>Tranquilizer</td>
<td>2001</td>
</tr>
<tr>
<td>Levomethadyl</td>
<td>Opiate Dependence</td>
<td>2003</td>
</tr>
</tbody>
</table>
Seldane, Poster Drug for TdP & SCD from Non-Cardiac Drugs

Terfenadine

Fexofenadine

• No preclinical signal; no effects on APD or in vivo QT
• 5 cases TdP/million pt-mo; f ~ 10^-6; undetectable in clinical trials; human QTc surrogate
• At 60 mg b.i.d., QTc increased 6 msec (~2%)
• Brown lab shows hERG to be molecular target for terfenadine risk

Normal sinus rhythm (hr= 66 bpm)

Torsades de Pointes

Courtesy of Dr. A. J. Moss

Vulnerable Period of a Cardiac Cycle
Modeling Cardiac Arrhythmias; Ion Channels, Cardiac Tissue, Whole Heart & Whole Humans

Adapted from Hoekstra et al, *Front Physiol*, 2012.
Essential Elements of Guidances

• **Nonclinical S7B 2005:** hERG, APD, ECG/QT; threshold values variable

• **Clinical E14 2005:** TQT, placebo & pos.ctrl, PK/PD, expensive, upper bound of 95% CI for $\Delta\Delta QT_c > 10$ ms biased to sensitivity vs specificity

• **CiPA:** shift from delayed repolarization to proarrhythmogenicity; replace TQT 07/15/15, revise S7B 07/16/16
A. Arrhythmias

Odds ratio 2/3 at best between hERG signal and Ventricular Arrhythmias, DeBruin et al, 2005

B. HERG and Delayed Repolarization (ChanTest)

<table>
<thead>
<tr>
<th></th>
<th>- HERG</th>
<th>+ HERG</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>9/118 (8%)</td>
<td>9/118 (8%)</td>
</tr>
<tr>
<td></td>
<td>False Neg</td>
<td>Concordant</td>
</tr>
<tr>
<td></td>
<td>80/118 (68%)</td>
<td>19/118 (16%)</td>
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<tr>
<td></td>
<td>Concordant</td>
<td>False Pos</td>
</tr>
<tr>
<td>+ Impaired Repol (APD/QT)</td>
<td>- Impaired Repol (APD/QT)</td>
<td></td>
</tr>
</tbody>
</table>

It’s NOT all about HERG!

e.g., pentobarbital, verapamil, tolterodine, ranolazine, vanoxerine

Drugs Affect Multiple Ion Channels: MI CE Hypothesis
Results of Present Guidances

• No withdrawals for QT-related arrhythmias since S7B & E14
• NDA submission of QT prolongers has been reduced

BUT

• Nonclinical signal levels are uncertain e.g., safety margins of hERG, APD & QT assays
• Clinical TQT strongly weighted to sensitivity vs specificity e.g., upper bound 95% CI \( \Delta \Delta \)'s 10 ms
• FDA dataset of 34 TQT studies showed 95% approval at 10-20 ms ↑ & 83% at > 20 ms
• Regulators approve drugs with QT liability where benefits outweigh risks
• TQT more honored in the breach than the observance
• Significant liabilities from cardiac safety studies
  – TQT costs
  – Delays in development
  – Labeling restrictions
  – Negative impacts on physician’s prescribing preferences
• E14 and S7B restrict selection of lead candidates, eliminate potentially useful drugs and reduce patient benefit
ChanTest Considerations

S7B & E14 unsatisfactory because:
1. $+\text{HERG} \neq +\text{DR}^*$ (~ 30% nonclinical discordances)
2. $+\text{QTc} \neq +\text{TdP}$ (millions of clinical discordances)
3. $+\text{HERG} \neq +\text{TdP}$ (qed; DeBruin et al, 2005)
4. $-\text{HERG} \neq -\text{TdP}$

Why not:

MICE vs HERG alone?
Proarrhythmogenicity vs $\uparrow\text{QTc}$?

$\text{DR}^* = \text{Delayed Repolarization (}\uparrow\text{QTc or APD}_{90})$

**Comparison of hERG IC₅₀s Among Manual & Automated Patch Clamp Platforms**

IC₅₀ (µM)

- Pimozide
- Tertafedrine
- E4031
- Chlorpromazine
- Diltiazem
- Ketamine
- Verapamil
- Fluoxetine
- Quindine
- Diphenhydramine
- Loratadine
- Erythromycin
- Solodol
Predicting TdP at ChanTest: HERG vs MI CE

IC$_{50}$s of 55 Drugs Included in Dataset for hERG, Cav1.2 & Nav1.5 Effects

MICE SaVeTy Curves as TdP Predictors

MI CE Model Beats HERG Model as TdP Predictor

Model 1

Model 5

\[ P(+Tdp) = \left[ 1 + \exp \left( \beta_0 - \beta_{hERG} \cdot \log \left( \frac{hERG \ IC_{50}}{ETPC} \right) \right) \right]^{-1} \]

\[ P(+Tdp) = \left[ 1 + \exp \left( \beta_0 + \beta_{CavD} \cdot \log \left( \frac{hERG \ IC_{50}}{Cav \ IC_{50}} \right) \right) \right]^{-1} \]

MI CE: Superior TdP Predictor

Model 5 wins

Victory by ROC curve

MI CE vs HERG Assays

- The *in vitro* hERG assay has ~ 70% accuracy for predicting TdP
- Type 1 errors are most frequent but Type 2 errors occur
- We proposed a Multiple Ion Channel Effects (MICE) assay as a better predictor
- We measured concentration-responses of hERG, hNav1.5 and hCav1.2 to 32 torsadogenic and 23 non-torsadogenic drugs from many chemotypes
- We used automated gigaseal patch clamp for throughput, accuracy, robustness and cost
- To compare hERG and MICE assays, binary TdP outcomes were interpreted with logistic regression models
- MICE models especially #5 significantly increased TdP predictivity and reduced Type 1 and 2 errors
- MICE turn-around times are 10-20x faster than manual patch clamp. Cost is *de minimus* compared to the gain in predictivity
Predicting Cardiac Safety: MICE Assay Should be the Preferred Assay for Nonclinical Cardiac Risk Assessment

**Conclusions:**
- TdP: MICE ~ 3X better than hERG as predictor
- MICE vs hERG halved false positives, reduced false negatives 6-fold
- TATs: MICE QPatch ~ 10x faster than hERG manPatch

Adapted from Kramer et al. 2013 Nat Sci Reports.
The Future: CiPA

A Comprehensive *in vitro* Proarrhythmia Assay to:

1. evaluate effects on multiple cardiac ion channels (QED in this presentation)
2. provide a more complete and accurate assessment of potential effects on human cardiac electrophysiology using SC-derived cardiomyocytes and *in silico* models of the ECG
3. focus on proarrhythmia rather than QT prolongation
Why CiPA?

S7B & E14 unsatisfactory because:
1. $+\text{HERG} \neq +\text{DR}^*$ ( ~ 30% nonclinical discordances)
2. $+\text{QTc} \neq +\text{TdP}$ (millions of clinical discordances)
3. $+\text{HERG} \neq +\text{TdP}$ (qed; DeBruin et al, 2005)
4. $-\text{HERG} \neq -\text{TdP}$

- CiPA recommends:
  MICE vs HERG alone
  Proarrhythmogenicity vs $\uparrow$ QTc

$\text{DR}^* = \text{Delayed Repolarization (}$\uparrow$\text{QTc or APD}_{90}$)
• **MICE**: gigaseal patch clamp methods reduce Type 1 and Type 2 errors (nonclinical discordance < 10%, Kramer et al 2013)

• Proarrhythmogenicity in human stem cell-derived cardiomyocytes tested by:
  1. MEA & 2D propagation
  2. xCELLigence and contractility;
  3. mPC & probability of EADs
**SC-Derived Human Ventricular Myocytes Studies with MEA & Impedance**

**MEA**

- QRS
- FPD
- Na⁺ Spike Amplitude
- T-wave
- Beat Period

**xCELLigence**

- DMSO
- Ouabain 10 nM

**MEA**: Measures extracellular field potentials yielding ECG-like recordings. Na⁺ spike amplitude, field potential duration (FPD), beat period/rate in stem cell-derived human cardiomyocytes.

**xCELLigence**: Measures electrical impedance changes resulting from myocyte contraction. Detects amplitude, rise and decay times, beat period/rate, cell index.
MEA: HERG Block Prolongs FPD

Moxifloxacin prolongs FPD and induces arrhythmic events.

A: Line graph (green line) shows percent change in FPD vs. [Moxifloxacin], µM.

B: Waveform comparison of baseline versus moxifloxacin treatment. Arrows mark the peak of each T wave (FPD). Early arrhythmic events were observed with 300 µM moxifloxacin after 10 minutes (white trace).
Implementing CiPA at ChanTest

Nonclinical Cardiac Safety Triage

**Comparator Study**
- ScreenPatch®
  - [hERG, Nav1.5, Cav1.2]
  - LR Model
- HERG-Lite®
  - hERG Trafficking
- Selective hERG block or trafficking deficit

**Lead Optimization**
- FastPatch
  - [hERG, Nav1.5, Cav1.2, KvLQT1, Kir2.1]
  - MICE Concentration-Response Quantitative Profiling
  - MICE Model
- Selective hERG Block

**hIPSC-CM Assays**
- xCELLigence®
  - Contractility
  - Toxicity
- ↓ Cell Index
- MEA
  - MICE FPD ("QT")
  - EAD/Arrhythmias
- EAD/Arrhythmias

**GLP hERG**

- Comparator Study
- Lead Optimization

- = “No go” outcome

*Human Induced Pluripotent Stem Cell-Derived Cardiomyocytes

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# CiPA at ChanTest

## FastPatch (QPatch) Cardiac Channel Panel Screen (with Positive Control)

<table>
<thead>
<tr>
<th>Channel</th>
<th># Test Articles</th>
<th>Concentrations</th>
<th>Replicates/Conc.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cav1.2 (L-Type)</td>
<td>1</td>
<td>4</td>
<td>3</td>
</tr>
<tr>
<td>hERG (IKr)</td>
<td>1</td>
<td>4</td>
<td>3</td>
</tr>
<tr>
<td>Nav1.5</td>
<td>1</td>
<td>4</td>
<td>3</td>
</tr>
</tbody>
</table>

## OPTION: SaVety™ Assessment

- Report includes a Torsade de Pointes (TdP) risk assessment
- Profiles of known compounds with similar TdP risk assessment scores
- Comparison to hERG alone

Number of Channels: 3

## Study Design

<table>
<thead>
<tr>
<th>Cells/Test System</th>
<th># Test Articles</th>
<th>Concentrations</th>
<th>Replicates/(n)</th>
</tr>
</thead>
<tbody>
<tr>
<td>MEA</td>
<td>9</td>
<td>5</td>
<td>4</td>
</tr>
<tr>
<td>xCELLigence</td>
<td>5</td>
<td>4</td>
<td>4</td>
</tr>
</tbody>
</table>

Test will include vehicle and positive controls
Achieve Expected Outcomes
Colleagues

ChanTest
- Jim Kramer
- Carlos Obejero-Paz
- Tony Lacerda
- Yuri Kuryshev
- Andrew Bruening-Wright
- Greg Luerman
- ChanTest Staff Scientists

Leadscope
- Glenn Myatt
- Ohio State University
- Joe Verducci