In Vitro Assessment to Replace the Clinical TQT Study: The Comprehensive In Vitro ProArrhythmia Assay (CiPA) Initiative

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Hot Topics Session
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**Goal:** Develop a new paradigm for cardiac safety evaluation of new drugs that utilizes high throughput methods and provides a more comprehensive assessment of direct proarrhythmic potential by:

- evaluating effects on **multiple cardiac ionic currents** beyond hERG and ICH S7B (inward and outward currents)

- provide a more **complete (and accurate) assessment** of potential effects on **human cardiac** electrophysiology

- focus on **Torsades de Pointes** proarrhythmia rather than QT prolongation
Background I. Human Ventricular Ionic Currents

- hERG current (IKr) only one of multiple currents that define ventricular repolarization

- Drug effects on multiple channels confound interpretation of QT prolongation risk due to hERG inhibition, and therefore misclassification of risk
Example: Delayed Repolarization with Dofetilide (hERG Block) Mitigated by Other Currents

Dofetilide + Nifedipine

Calcium current block (Nifedipine) mitigates delayed repolarization with Dofetilide (hERG block)

Dofetilide + Lidocaine

Sodium current block (Lidocaine) mitigates delayed repolarization with Dofetilide (hERG block)

Martin et al., JCVPharm., 2014
Background II. Proarrhythmic Vulnerability and Early Afterdepolarizations (EAD’s)

Proarrhythmic vulnerability linked to impairment of repolarization and repolarization instability culminating in early afterdepolarizations (EAD’s)

- EAD’s are triggers for Torsades de Pointes arrhythmia
- Ease of EAD induction reflects proarrhythmic vulnerability
- Provide means of ranking proarrhythmic potential
Links: Delayed Repolarization, EAD’s and Torsades-de-Pointes Proarrhythmia

A.
- Increased QTc

B.
- Altered QTc
- Reentry

C.
- Early Afterdepolarization (EAD)
- Triggered Activity
- Torsade de Pointes Ventricular Fibrillation

adopted from Woosley, 1999
### Assays and Approaches Considered for Comprehensive Assay (In Order of Complexity, Integration)

<table>
<thead>
<tr>
<th>Approach</th>
<th>Description</th>
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<tr>
<td><strong>QSAR</strong></td>
<td>Models describing relationship between molecular structural features and properties or activities at given pharmacological/toxicological endpoint</td>
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<td><strong>Receptor Affinity Assays</strong></td>
<td>Typically competitive binding studies to ion channels</td>
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<td><strong>Single Channel Recording</strong></td>
<td>Highly detailed measure of current through a single ionic channel</td>
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<td><strong>Macroscopic Ionic Currents</strong></td>
<td>Detailed analysis of drug effects on functional cardiac currents; widely accepted</td>
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<td><strong>Isolated Cardiac Myocytes</strong></td>
<td>Cardiocytes of human origin more likely to reflect native physiology; availability of stem-cell cardiocytes vs. tissues</td>
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<td><strong>In vitro/in vivo proarrhythmia</strong></td>
<td>Tissues/organs or whole animal models mimicking enhanced proarrhythmia risk</td>
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<tr>
<td><strong>Computer Models of Cardiac Myocytes</strong></td>
<td>Reconstruction of electrical activity of ventricular myocytes from channel effects (delayed repolarization and EAD's)</td>
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<td><strong>Whole Heart Computer Models</strong></td>
<td>Reconstruction of ECG and drug effects (incorporates individual channels and action potential studies)</td>
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CiPA: Two Component Proposal

Ionic Currents / In Silico Based Approach

- Effects on Multiple Cardiac Currents (Voltage Clamp Studies)
- Reconstruction of Cellular Electrophysiology (In Silico Studies)

Myocyte-Based Approach

- Effects on Human Ventricular Myocytes (In Vitro Studies)

- Complementary approaches
- Not designed to reproduce arrhythmia
Core *In Vitro* Strategy. **Voltage Clamp Studies**

**Ionic Currents**

- **Voltage clamp studies**
  - Effects on cardiac currents
  - Human channels in heterologous expression systems
  - Establish best practices, **standardization across assays, laboratories**

- **Higher throughput automated patch platforms**
  - Newer approach provide sufficient **sample size and statistical power** to enable *in silico* studies
  - Efficiently determine **basic characteristics** of drug effects on currents for *in silico* reconstruction
Likely Candidate Currents

- $i_{Kr}$ (hERG) – delayed ventricular repolarization
- $i_{Na_{fast}}$ (Nav1.5) – excitability, conduction
- $i_{Na_{late}}$ (Nav1.5) – repolarization, mitigate hERG block
- $i_{Ca_L}$ (Cav1.2) – A-V conduction, mitigate hERG block
- $i_{Ks}$ (KvLQT1-minK) – delayed ventricular repolarization
- $i_{K1}$ (Kir2.1) – excitability, conduction, repolarization
Core *in silico* Strategy: Reconstruction of the Cardiac Action Potential

*In Silico* Reconstruction of Action Potentials

- Global effects on repolarization based on multiple ion channel effects
- Approach based on link between delayed repolarization supporting early afterdepolarizations (EAD’s) and TdP
In Silico Strategy: Example of iKr (hERG) Block

Parameters of Interest:
- Action potential duration
- EAD-like activity
- Others (under discussion)

Core *In vitro* Strategy: Human Cardiomyocytes

**Electrophysiologic studies:**
- **Human stem-cell derived ventricular cardiomyocytes;** well characterized, physiologic recording conditions
  - Action potential studies, **focus on repolarization** (duration, early afterdepolarizations, cellular integration)
  - Reproducibility essential, robust validation
  - Confirm drug effects from voltage clamp/*in silico* reconstructions
Stem-Cell Derived Myocytes: Possible Experimental Approach

Field Potential Measures (Microelectrode Array Techniques)

E-4031: Concentration-dependent Block of iKr Delays Repolarization, Provokes EAD’s

Nifedipine: Concentration-dependent Block of IcaL Speeds Repolarization
Approach based on

a) mechanistic understanding, integrating effects on multiple ion currents with *in silico* reconstruction

b) confirmation in human ventricular myocyte-based assay

- Not typical preclinical assay based on binary discrimination in complex, integrated (poorly understood) biological system

- Need input from safety pharmacologists, electrophysiologists, computational modelers, cell biologists, regulators

**First Steps:** Seek Input, Establish Workstreams (ongoing)
Comprehensive *In Vitro* ProArrhythmia Assay (CiPA)

**What It Will Do:**
- Standardize *in vitro* assays (used to characterize drug effects) and *in silico* modeling of drug effects
- Define role of human cardiomyocytes to inform on proarrhythmic potential of drugs
- Provide proarrhythmic ranking based on calibration efforts with agreed-upon standards

**What It Will Not Do:**
- Maintain regulatory status-quo for an imperfect surrogate marker of proarrhythmia
Backup
Integrating Ionic Current Effects: Core Component (I)

**In Silico Reconstruction of Action Potentials**

- Integrated effects based on drug actions on multiple ion channels
- Approach based on link between delayed repolarization supporting early afterdepolarizations (EAD’s) and Torsades
- Ability to elicit early- (or delayed) afterdepolarizations, reduced maximum upstroke velocity