Comprehensive ProArrhythmia Assay Schema

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for the Comprehensive in Vitro ProArrhythmia Assay Group.
**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** (inward and outward currents)

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

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

- Present **QT focus** results in unwarranted drug attrition, misclassification of hazard and risk

- Drug effects on multiple channels confound interpretation of QT prolongation risk due to hERG inhibition, and therefore misclassification of risk

- Early, rapid, comprehensive survey of ion current effects linked to human ECG desirable
**Assumption:** proarrhythmic vulnerability linked to impairment of repolarization that supports instability or early afterdepolarizations (EADs) during the action potential

- EAD’s a manifestation of proarrhythmic vulnerability
- Provide means of ranking proarrhythmic potential
- Electrophysiologic heterogeneity supports EAD initiation
## Assays and Approaches Considered
(In Order of Complexity, Integration)

<table>
<thead>
<tr>
<th>Approach</th>
<th>Description</th>
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<tbody>
<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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<tr>
<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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<tr>
<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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Comprehensive Proarrhythmia Assay Proposal: Two Component Paradigm

Ionic Currents-Based Approach

- Effects on Multiple Ionic Currents
- \textit{In Silico} Reconstruction - Integrated Cellular Effects

Cell-Based Approach

- Effects on Human Ventricular Myocytes

- Parallel assessment of integrated drug-induced effects
- \textbf{Hazard/risk identification for drug candidates}
- Not designed to reproduce arrhythmia
Core In Vitro Strategy. Voltage Clamp Studies

Ionic Currents in Heterologous Expression Systems

• Voltage clamp studies
  – Standardized voltage clamp protocols, conditions; establish best practices
  - Reduce variability, establish best practices allow comparisons across assays and laboratories
  – Allows for decisions in a standardized, unbiased manner

• Higher throughput automated patch platforms
  - Provide sufficient sample size and statistical power to confidently parameterization models
  - Determine potency (IC50), (voltage- and use-dependence?)
  - Provide basic characteristics of drug effects on currents needed for in silico reconstruction
Candidate Currents

- iKr (hERG) – delayed ventricular repolarization

- INafast (Nav1.5) – excitability, conduction

- INalate (Nav1.5) – repolarization, mitigate hERG block

- ICaL (Cav1.2) – A-V conduction, mitigate hERG block

- IKs (KvLQT1-minK) – delayed ventricular repolarization

- IK1 (Kir2.1) – excitability, conduction, repolarization
Integrating Ionic Current Effects: Core Strategies

I. In Silico Reconstruction of Action Potentials

- Global effects on repolarization based on multiple ion channel effects
- Ability to elicit early- (or delayed) afterdepolarizations, reduced maximum upstroke velocity
- Approach based on link between delayed repolarization supporting early afterdepolarizations (EAD’s) and TdP
- Electrophysiologic model(s) to be determined
- Comparison with human ECG’s to test accuracy of cellular action potential reconstruction
- Potential for future whole-heart modeling
- Models of phenotypically immature stem-cell derived cardiocytes may be “corrected” for ion current characteristics, densities
In vitro Cellular Integration: Core Strategies

II. Effects on Human Ventricular Myocytes
- Well characterized human stem-cell derived cardiomyocytes, physiologic recording conditions
- Action potential studies, focus on repolarization (duration, early and delayed afterdepolarizations)
- Physiologic recording conditions
- Robust validation, reproducibility necessary
Validation Efforts and Paths Forward

Approach based on **mechanistic understanding of integrated effects on multiple ion current linked to proarrhythmia**

- hERG alone is incomplete; other currents influence effects
- QT prolongation not always proarrhythmic (e.g., small effects)

**Multiple approaches inform on integrated effects**

- Proarrhythmic vulnerability linked to impaired repolarization that supports abnormal early activity during repolarization

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

- Input required from industry, academics, regulators

**Transformational, Mechanistic-Based In-Vitro/In Silico Approaches to Assess Proarrhythmic Risk**