Comprehensive ProArrhythmia Assay Schema

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Comprehensive *In Vitro* ProArrhythmia Assay

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

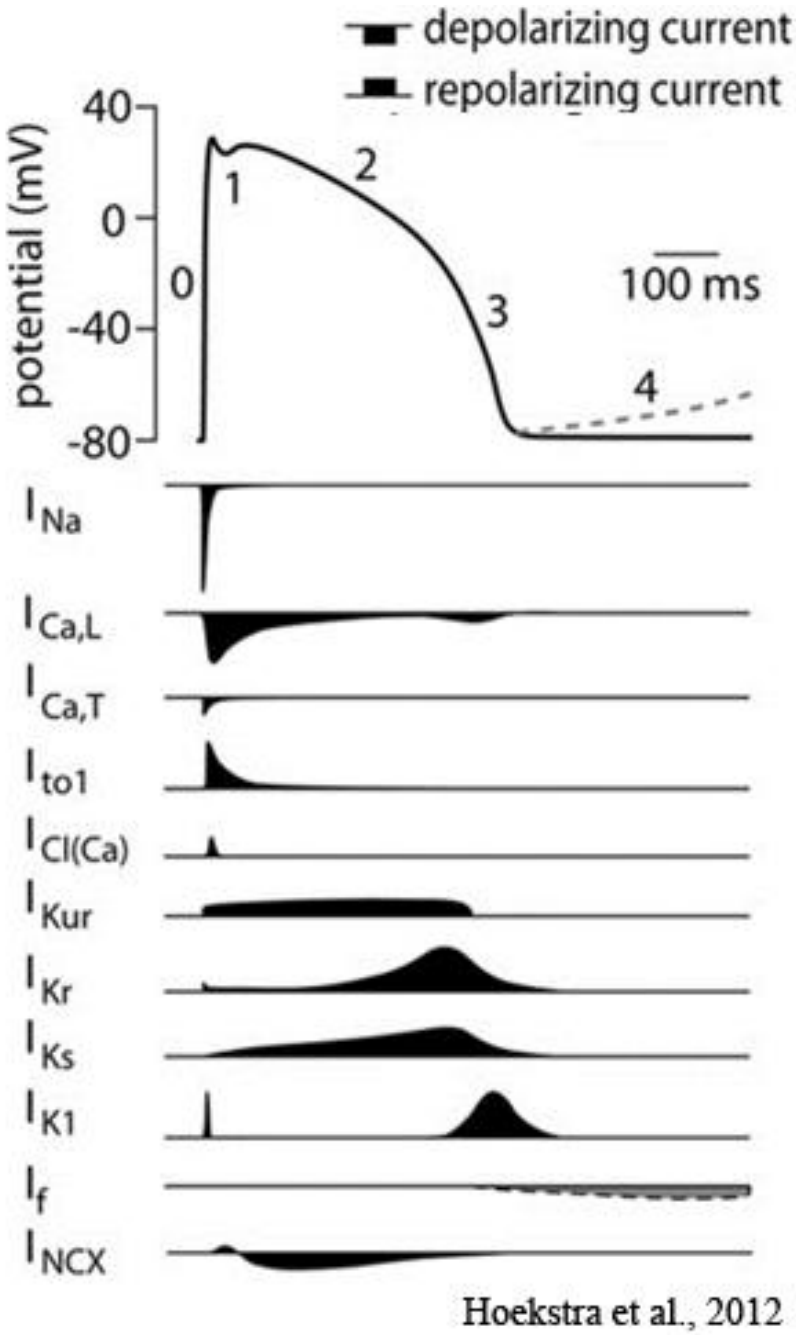
- focus on **proarrhythmia rather than QT prolongation**

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



Hoekstra et al., 2012

Background II. Proarrhythmic Vulnerability and Early Afterdepolarizations (EAD's)

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)

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

Comprehensive Proarrhythmia Assay Proposal: Two Component Paradigm

Ionic Currents-Based Approach

Effects on Multiple Ionic Currents

+

***In Silico* Reconstruction - Integrated Cellular Effects**

Cell-Based Approach

Effects on Human Ventricular Myocytes

- Parallel assessment of integrated drug-induced effects
- **Hazard/risk identification for drug candidates**
- Not designed to reproduce arrhythmia

Core *In Vitro* Strategy. Voltage Clamp Studies

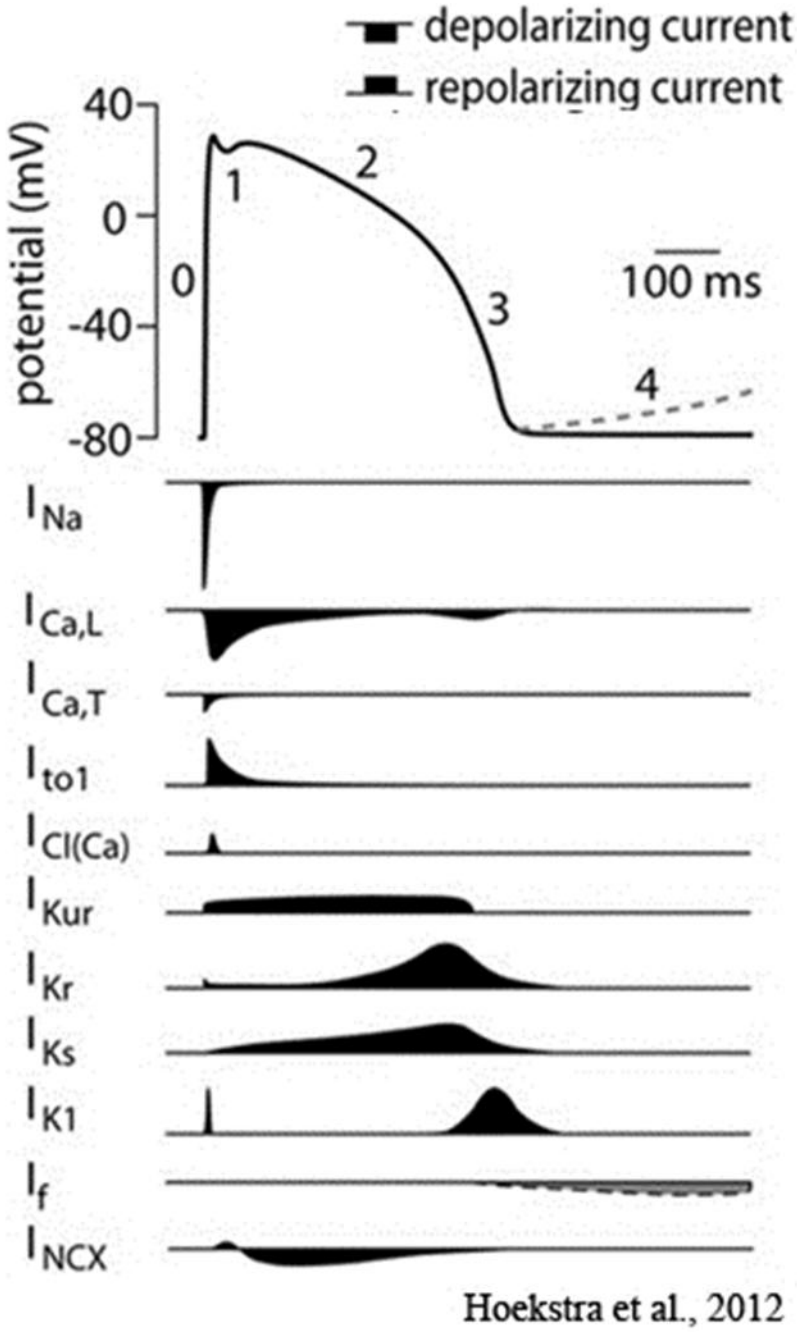
Ionic Currents in Heterologous Expression Systems

- **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