The *In Vitro* Evaluation of Proarrhythmic Risk — The Evolving CiPA Paradigm

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Outline

• Introduction and Rationale for CiPA
  ▪ Definition
  ▪ Justification, Need, Time
  ▪ Overarching Goals

• Components
  ▪ Ionic Currents
  ▪ In Silico Reconstruction
  ▪ Stem-Cell Derived Cardiomyocytes

• Challenges, Progress and Timelines

• Conclusions
**Goal:** Develop a **new in vitro paradigm** for cardiac **safety evaluation** of new drugs that provides a more accurate and comprehensive mechanistic-based assessment of proarrhythmic potential.

- Focus on **proarrhythmia (not QT prolongation)** to improve specificity compared to preclinical hERG and clinical TQT studies.

**How?**
- Define drug **effects on multiple human cardiac currents**, 
- Characterize **integrated electrophysiologic response** using **in silico reconstructions** of human ventricular electrophysiology, 
- Verify effects on human stem-cell derived ventricular **myocytes**
CiPA: Background and Established Need (1)

- 6 drugs removed from US marketing (1991-2006) due to QT prolongation leading to Torsade-de-Pointes (TdP)
  - Rare but potentially lethal ventricular arrhythmia

- Reduced potassium repolarizing current iKr (Kv11.1, encoded by hERG gene) linked to congenital LQT2 and most drugs that prolong QT interval (ex: terfenadine)

- hERG becomes early (and convenient) proarrhythmia “gatekeeper”
  - Availability of transfected cell lines; binding/current measures
  - Advances in automated planar patch techniques
  - Plentiful commercial services
  - Proposed 30-fold margin for cardiac safety (IC50 hERG block/effective therapeutic free plasma conc.), Redfern, 2003
  - ICH S7B Guidance (May 2005)
"hERG-centric" ICH S7B approach “successful”
- No drugs removed from marketing due to TdP risk since 2003
- Clinical ICH E-14 Guidance (TQT study) also played a role

HOWEVER:
- hERG not very predictive (“throw baby out with bathwater”)
- *in vivo* animal assays not always predictive of human QT findings
Marginal hERG Assay Performance (vs. TQT Prolongation, a Surrogate Marker of TdP)

- Mean QTc Prolongation (TQT Studies) vs. hERG “Safety Margin” (IC50/[free plasma])

- Only some drugs “obey” 30-fold Safety margin (not 14, 18, 6)

- QT prolongation not equivalent to TdP; a surrogate marker

- Thesis: many good drugs eliminated early in drug discovery due to hERG (unwarranted attrition)
Why is hERG Insufficient to Predict Delayed Repolarization?

REPOLARIZATION: *integrated response of multiple currents*

DELAYED REPOLARIZATION: Reduced *NET OUTWARD CURRENT*
- block of iKr current/hERG
- enhanced inward current (Na⁺, Ca++) during plateau (phases 2, 3)

Balance of inward and outward human currents that define drug effects on ventricular repolarization.

Present focus on (iKr, hERG) results in premature and unwarranted drug attrition, misclassification of risk.
Drugs: Multiple Cardiac Currents are Affected

- Lack of selectivity of drugs on cardiac currents affecting (depolarization and repolarization) necessitates consideration of multiple currents
- Automated voltage clamp data essential for timely and economical evaluation of multiple currents
Many Drugs Affect Multiple Cardiac Currents: Importance of Multichannel Block

- Multi-channel block: define overall drug response

- Verapamil: drug eliciting multi-channel block but minimal/no QT, no TdeP (hERG block mitigated by calcium current)

- Experiment (right): block of outward iKr current (hERG) by dofetilide reversed by block of inward calcium current by nifedipine

- Need for more comprehensive assessment of effects on multiple human currents (integrated human systems)

Martin et al., J Cardiovasc Pharm., 2004
Need For a More Comprehensive PROARRHYTHMIC Assessment

- Drugs recognized to block hERG but not delay repolarization or prolong QT (verapamil, fluoxetine, ranolazine, pentobarbital)

- Regression techniques predict proarrhythmia from *in vitro* studies using 3 currents (iKr, ICa, INa) better than hERG alone (Kramer et al, 2013)

- *In silico* reconstructions show that hERG block may be mitigated by other (e.g. calcium) channel block (Mirams et al., 2011)

- TQT Study Results: Often difficult to interpret “low-signal” risk
  - “Marginal” QT prolonging drugs (e.g. 8 msec ↑)
  - Drugs that show small, “plateau” response for QTc↑
  - Drugs with “autonomic effects” that affect HR, BP
Cellular Proarrhythmia: Abnormal Emergent Responses that affect Repolarization (not simply ↑APD or ↑QTc)

Focus on proarrhythmic vulnerability: impaired cellular repolarization, electrical instability

- In extreme form, instability manifest as early after-depolarizations (EAD’s) associated with initiation of proarrhythmia

- Impaired repolarization used to rank proarrhythmia based on comparisons with known TdP drugs

Proarrhythmic Liability (an Integrated, Emergent Effect) Manifest on Cellular Level With Human Responses
Comprehensive *In Vitro* Proarrhythmia Assay: Three Scientific Core Pillars

**Drug Effects on Multiple Human Cardiac Currents**

- $I_{Na}$
- $I_{Na,L}$
- $I_{Ca,L}$
- $I_{to1}$
- $I_{Kr}$
- $I_{Ks}$
- $I_{K1}$

modified from Hoekstra et al., 2012

**In Silico** Reconstruction Human Ventricular Cellular Electrophysiology

\[ I_{stim} = C \frac{dV_m}{dt} + I_m \]

**In Vitro** Effects Human Stem-Cell Derived Ventricular Myocytes

Preclinical ECG & Phase 1 ECG Studies: Complementary Data
Core Component I. Human Ionic Currents, Voltage Clamp Studies, Heterologous Expression

- **Ion Channel Working Group (SPS):** develop protocols, preliminary hERG assay tested

- Standardized voltage clamp protocols to establish best practices, reduce bias and variability, allow comparisons of automated platforms across laboratories

- Information on kinetics-, voltage- and use-dependence to parameterize models (hERG essential)

Robust characterization of drug effects on human currents enables *in silico* reconstructions of integrated responses
- **In Silico Group (FDA):** Drug effect multiple human currents integrated to describe cellular electrophysiologic effects

- Ability to elicit changes in repolarization instability, early afterdepolarizations, reduced upstroke velocity using select model (modified O’Hara-Rudy model considered)

- Ranking of integrated responses compared with clinical examples of different TdP liabilities
Core Component III: *In vitro* Effects, Human Stem Cell-Derived Ventricular Cardiomyocytes

- **Myocyte Group (HESI):** Verification of *in silico* reconstructions with well characterized human stem-cell cardiomyocytes

- Inform on repolarization effects not anticipated from ion channel and *in silico* reconstruction efforts
Clinical Foundations: Compound Selection & Clinical Regulatory Group

- **Compound Selection/Regulatory (CSRC):**
  - Categorization available drugs for proarrhythmic risk:
    - High, Intermediate, Low risk categories
    - Evaluate clinical data for proarrhythmia
    - Overall experience, history, patient population, pharmacokinetics
    - 29 drugs nominated for model development and verification

- Input from clinical developers/regulators
  - Validation & acceptance of CiPA paradigm

- Define Phase 1 ECG Verification Efforts
Core Component I. Human Ionic Currents, Voltage Clamp Studies: Progress

Proposed two sets of protocols for 7 ion channels

- “Dynamic block” protocols: (IC50, kinetics and state-dependence) under consideration; voltage clamp

- “Physiologic protocols”: voltage-rate- and use-dependence under consideration

hERG block protocols tested for dofetilide, cisapride, verapamil: concentrations, temperature (RT vs 37oC),

Data provided to In Silico Working Group to use for training in silico model; Protocols and datasets to be made available in 2015
Core Component II.

Computer Reconstructions: Progress

Initial Test Case: D,l Sotalol (known torsadogen)
- Recognized by CiPA Compound Selection Working Group
- Product labeling for QTc prolongation and TdP

- O’Hara-Rudy model recapitulates delayed repolarization in human ventricular tissues based on IC50s values for block of hERG, Cav1.2 and Nav1.5 (Kramer et al. (2013))

- Not replicated by Grandi or ten Tusscher in silico models

- EAD’s not generated in either Ten Tusscher or Grandi models with prominent iKr/hERG block
Core Component III. Stem-cell Derived Cardiomyocytes: Progress

Pilot Study Completed:

12 Sites, 2 approaches:
- Multi-electrode arrays (MEA-8 sites)
- Voltage-sensing optical (VSO-4 sites)
- Dyes or integral sensing proteins
- Myocytes prepared according to vendor specifications
- Protocols “common” as appropriate

8 compound test set (blinded)
  4 drugs: calibrate iKr, iT, INaF, ICaL
  4 drugs: preliminary test set
  4 concentrations; 3 cells tested

Prelim. analysis; concordance within VSO; MEA started
Validation study for proarrhythmic risk prediction (MHLW Grant for Nat. Inst Health Sciences Japan)

Industry-Government-Academia Consortia

Define utility of human stem cell derived cardiomyocytes for detecting proarrhythmia

- Standardized protocol: high density sheet, iCell cardiomyocytes studied with MEA system (MED64)
- Endpoints: ↑field potential duration, EAD/triggered activity
- Stage 1: E-4031, cisapride, chromanol293b effects, 4 sites
- Results: common protocol and training ensures high degree of reproducibility (Nakamura et al., J Pharm Sci, 2014)
Group Efforts Against Timelines

Parties Involved:

Cardiac Safety Research Consortium: Cmpd Selection Team

FDA: In Silico Working Group

ILSI-HESI: Myocyte Stem Cell Working Group

Safety Pharm. Society: Ion Channel Working Group

Regulators: FDA, Health Canada, EMEA, Japan

Japanese Safety Pharmacology Society, JiCSA

Academic Contributors

Vendors and Suppliers

Timelines: Progress Review: 1Q 2016
Summary: CiPA

**What It Is:** Proposal to evaluate proarrhythmic risk based on mechanistic electrophysiologic understanding of proarrhythmia with two primary components

I. *In vitro* drug effects, multiple cardiac channels
   + *In silico* reconstruction of electrical effects

II. Confirmation using human stem-cell derived cardiomyocytes

**What It is Not:** Approach that negates well-controlled preclinical *in vivo* ECG assessment in preclinical studies
Comprehensive *In Vitro* ProArrhythmia Assay (*CiPA*)

**What It Will Do:**
- Prevent early (often unwarranted) attrition due to early testing for hERG liabilities with updated technologies and knowledge of proarrhythmia
- Provide a more complete assessment of proarrhythmic risk (rather than surrogate QT prolongation alone)
- Replace TQT study (high sensitivity) for higher specificity, less “false positives” functional hERG studies
- May “rescue” drugs mislabeled with risk warnings by small degrees of QT prolongation in TQT studies

**What It Will Not Do:**
- Not replace need for careful assessment of QT effects in phase 1 ECG safety studies (eQT studies/conc.-response modeling)
What It Is: Reflection of evolving practices by some Pharma for early in vitro detection of QT prolongation
- Regression techniques predict proarrhythmia from in vitro studies of 3 currents better than hERG alone (Kramer et al, 2013)
- In silico reconstructions show that hERG block may be mitigated by other (e.g. calcium) channel block (Mirams et al., 2011)
- Recognition that hERG represents only one of multiple ion currents defining repolarization (a surrogate marker of proarrhythmia)

What It Is Not: Novel approach never considered or employed by pharma, academics as part of exploratory/frontloading safety studies
Comprehensive *In Vitro* ProArrhythmia Assay (*CiPA*)

**What It Is:** Proposal to be developed by numerous stakeholders (Regulators, Pharma, Academics, CRO’s)
- An evolving initiative with evolving workflows in need of wide participation and input of multiple parties
- A proposal that needs to be qualified

**What It is Not:** Predetermined or predefined regulatory schema with undue influence of vendors
- An approach that will negate other experimental approaches for internal decision-making (e.g., binding studies, tissue-based or *in-vivo* studies, other informative alternatives)
Comprehensive *In Vitro* ProArrhythmia Assay (*CiPA*)

**What It Will Do:**
- Standardize *in vitro* assays used to characterize drug effects, standardize *in silico* models, establish best practices for stem-cell derived cardiomyocyte models (comparable to “acceptable” TQT studies evolved)
- Provide proarrhythmic ranking based on calibration/validation efforts with agree-upon standards
  – Likely lead to revision of S7B, E-14 guidelines, more sophisticated and realistic modeling of drug effects

**What It Will Not Do:**
- Maintain regulatory status-quo for an imperfect surrogate marker of proarrhythmia
- Replace biological studies employing fully integrated systems
CiPA: proarrhythmic risk based on mechanistic understanding of integrated, cellular emergent drug effects on multiple human cardiac currents

- hERG (iKr) block alone is insufficient
- QT prolongation not always proarrhythmic
- Proarrhythmic vulnerability linked to impaired repolarization that supports abnormal electrical activity
- Not typical preclinical assay reliant on complex, poorly understood system, binary output

CiPA Expectations:
- reduce unwarranted attrition early candidates
- enable progression of low risk TdP drugs to phase 1 studies (and confirmatory clinical ECG findings)
Thanks for your attention.