In silico assessment of drug cardiac safety and efficacy: state of the art and interaction with the *in vitro* world

Trial lecture for the degree of Philosphae Doctor

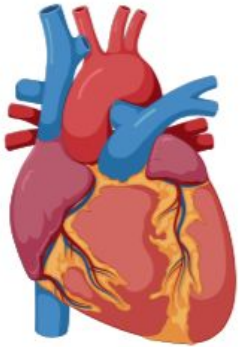
Márcia Vagos

Simula Research Laboratory
Computational Physiology Department

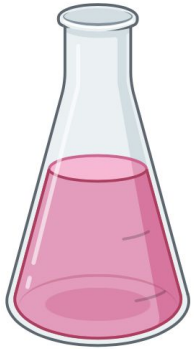
UiO : University of Oslo

Outline

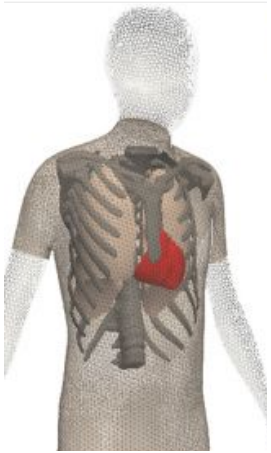
1. Principles of cardiac electrophysiology and drug safety



2. Common *in vitro* assays to evaluate drug safety

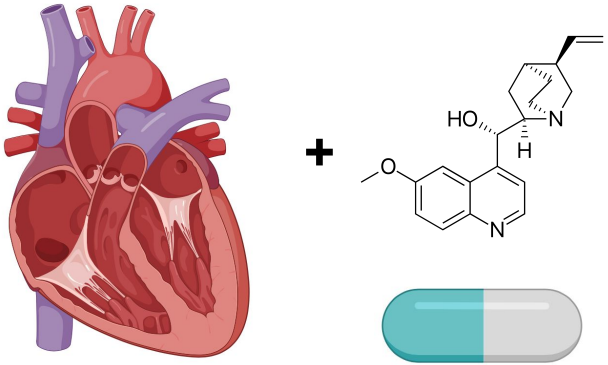


3. Novel *in silico* modeling approaches



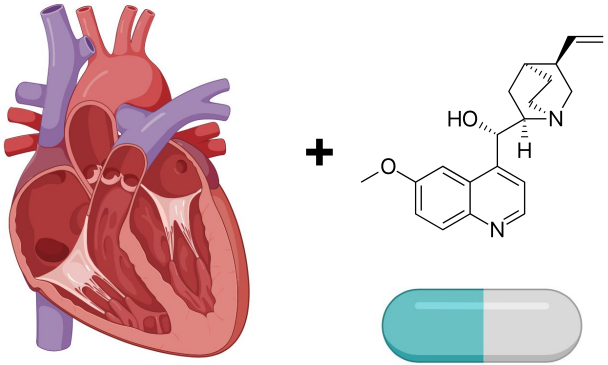
https://www.cs.ox.ac.uk/insilicocardiotox

Several health conditions are treated with pharmaceutical compounds



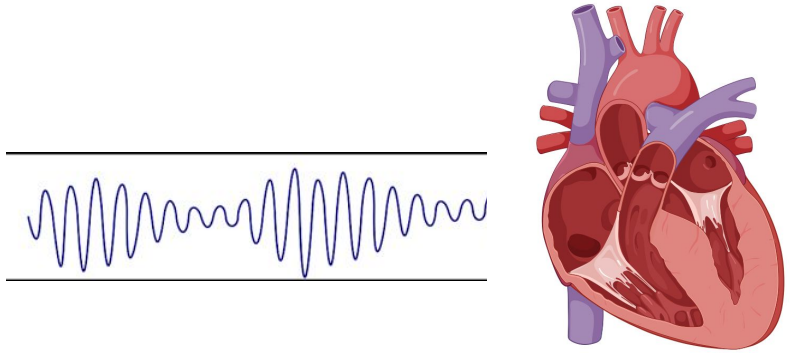
- control heart rate
- improve contractility
- enhance electrical activity

Several health conditions are treated with pharmaceutical compounds



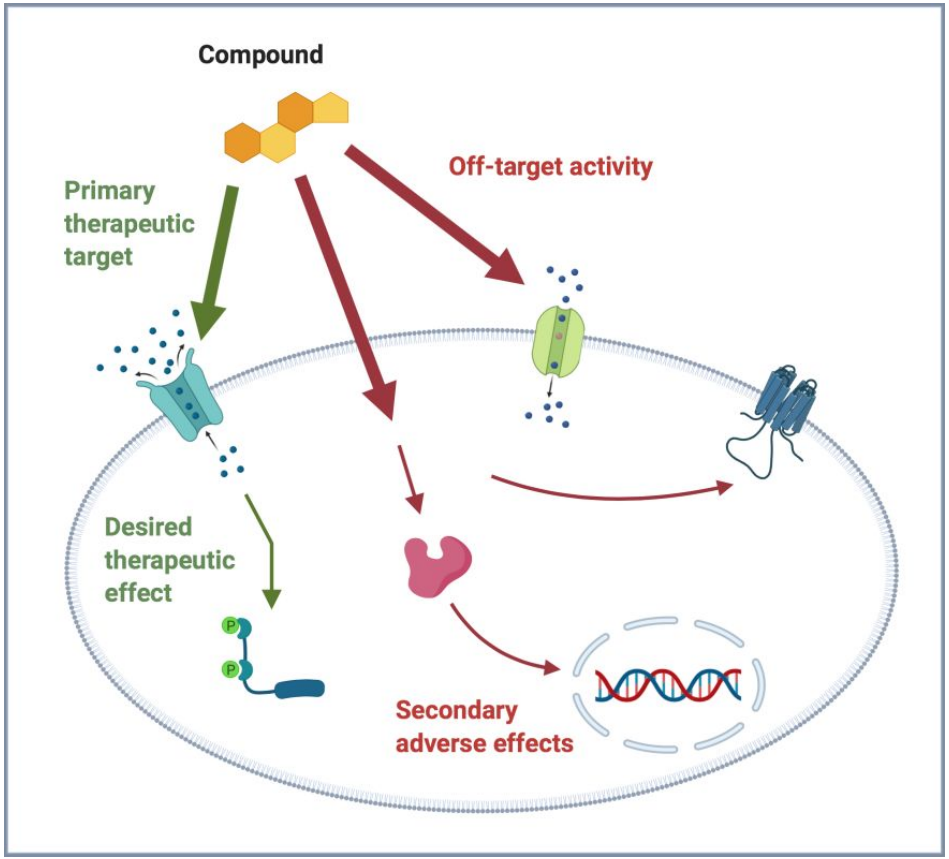
- control heart rate
- improve contractility
- enhance electrical activity

Drugs can have detrimental effects on cardiac function



- cardiotoxicity
- arrhythmias
- cardiac muscle dysfunction

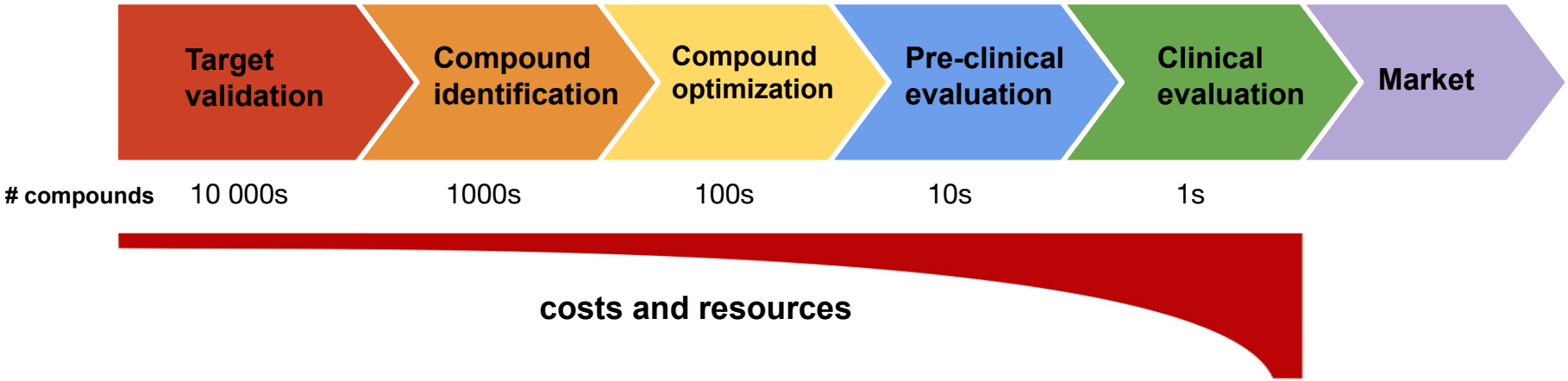
Safety pharmacology is concerned with the assessment of unwanted side effects of drugs



Need for a thorough assessment of a compound's safety and cardiotoxicity

Cardiac safety pharmacology

Drug development is a long and costly process



→ cost per new drug reaching the market estimated at **$1 billion**

→ ~ **10 years** of development from drug synthesis to market approval

→ **>500 000** animals used in tests per year

→ **20-50%** of all advanced candidates are abandoned due to adverse outcomes

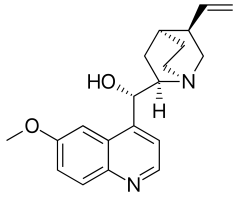
Adapted from Davies et al., *Drug Discovery Today* 21 (6), 2016

A thorough characterization of new drugs is required during development

Pharmacodynamics

Pharmacokinetics

Drug interactions

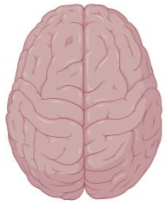


Chemistry

Pharmacology

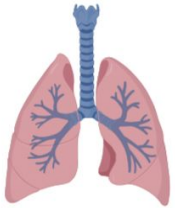
Toxicology

Safety Pharmacology 'Core Battery Studies'



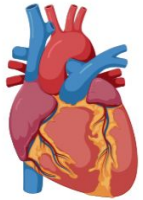
CNS

- sensory/motor
- behavior
- reflexes



Respiratory

- respiratory rate
- tidal volume
- O_2 saturation

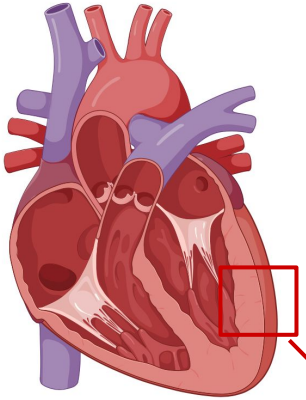


Cardiovascular

- heart rate
- repolarization (APD)
- hERG inhibition
- conduction

Adapted from Pugsley et al., BJP, 2008

How do drugs interact with cardiac electrophysiology?



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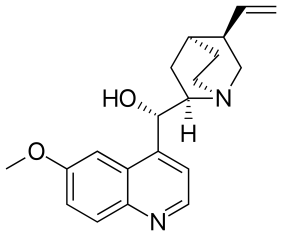

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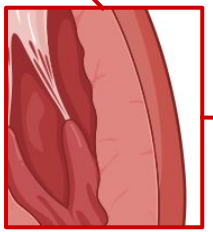
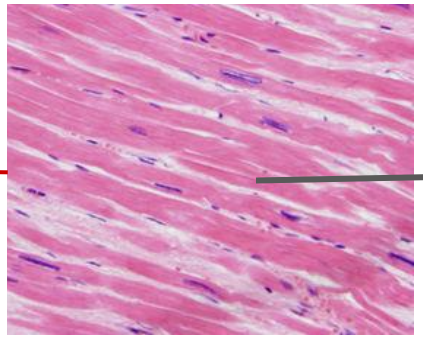
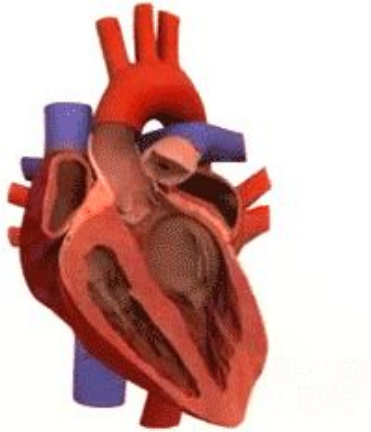
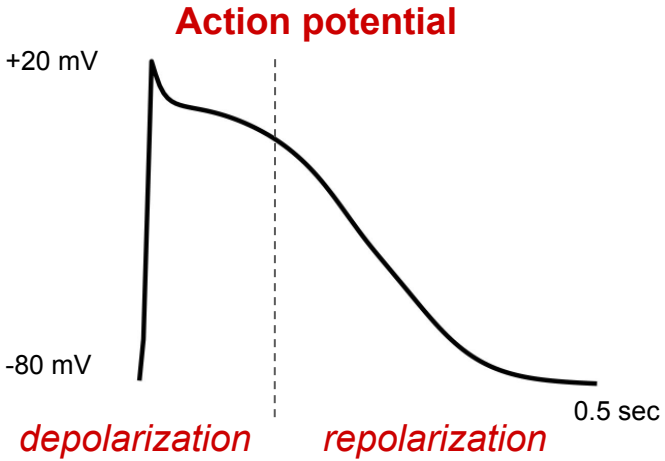


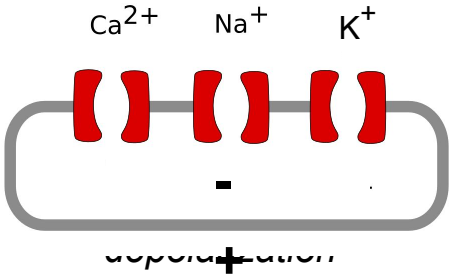


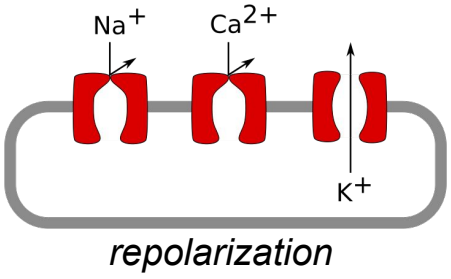
cardiomyocyte
(CM)

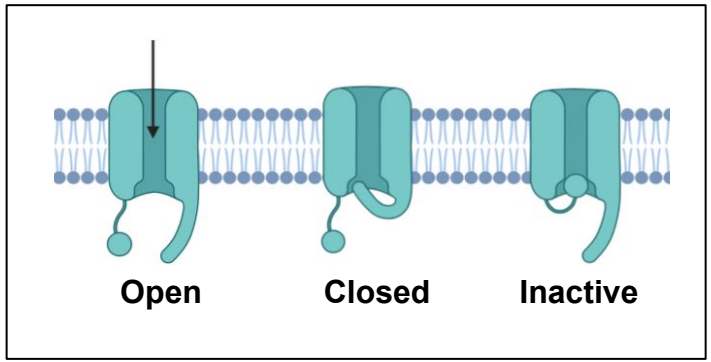


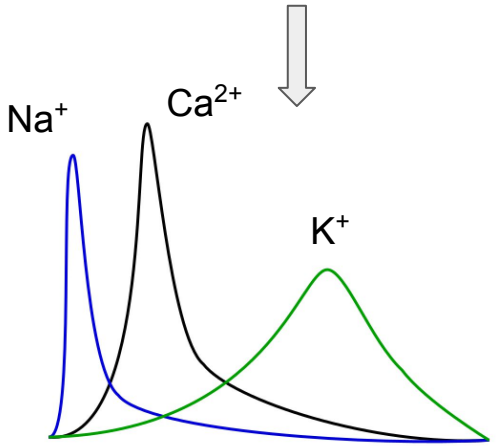
Ionic currents through the cell membrane give rise to action potentials (AP)



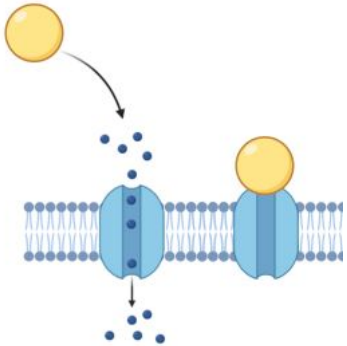






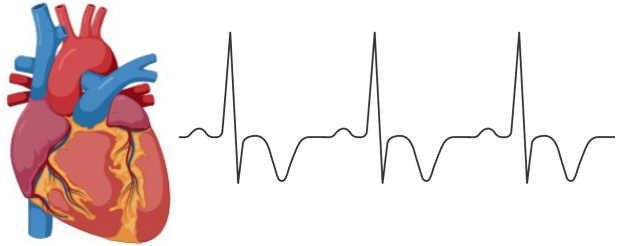


Drugs affect cardiac electrophysiology by interaction with ion channels

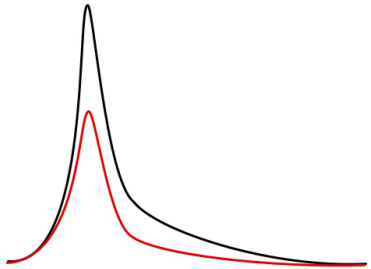


Drug block interacts with ion channel, altering the ionic current

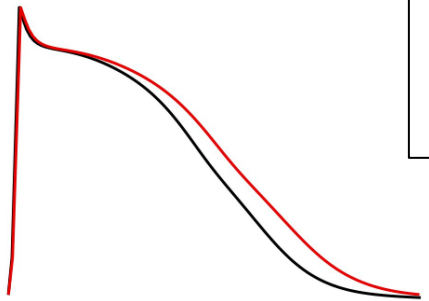
Changes in cardiac function



current density



ionic current



action potential

Experimental data on drugs effects is obtained with *in vitro* assays

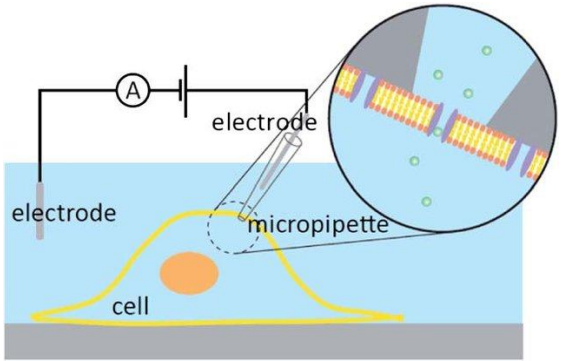
Ionic currents

- Manual patch clamp (gold standard)
- High throughput screening (HTS)
  - Automated patch clamp
  - Ca^{2+} fluorescence imaging

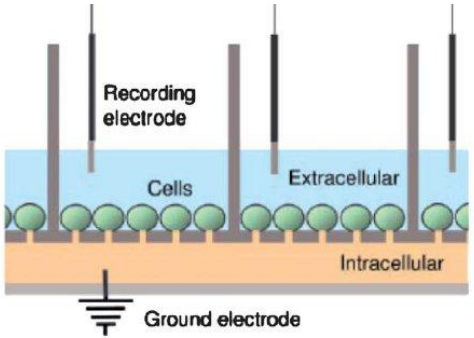
Action potentials

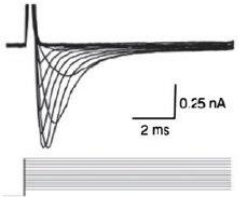
- Sharp electrode

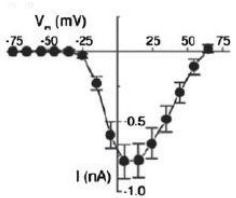
Whole-cell patch clamp



Automated patch clamp



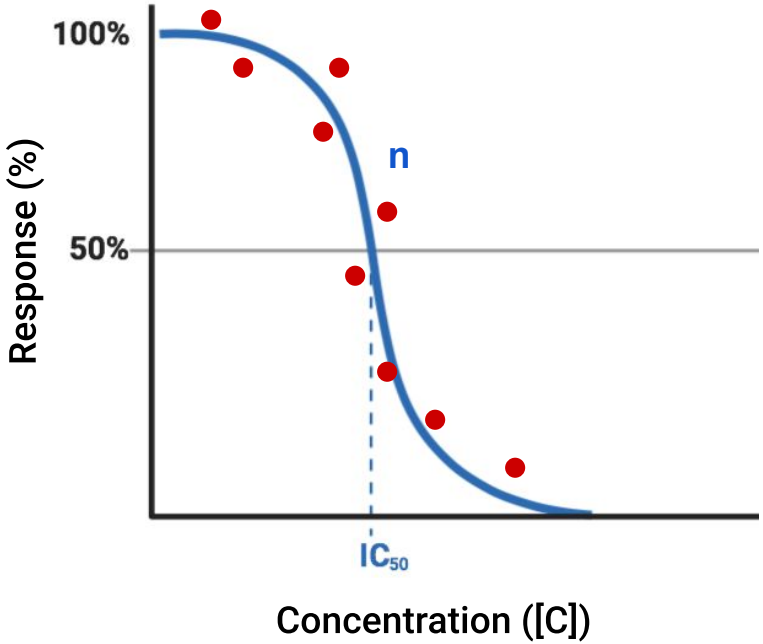




Demarche et al., Techniques for recording reconstructed ion channels, The Analyst 136(6), 2011

Patch clamp assays provide data on concentration-dependent drug effects

‘concentration-effect’ (CE) curves of ion-channel inhibition



Hill function

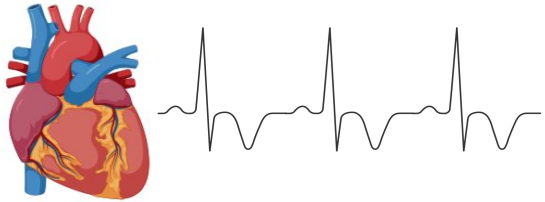
$$R([C]) = \frac{1}{1 + \left(\frac{[C]}{[IC_{50}]}\right)^{n}}$$

IC50 - drug concentration at which 50% of maximal response is observed

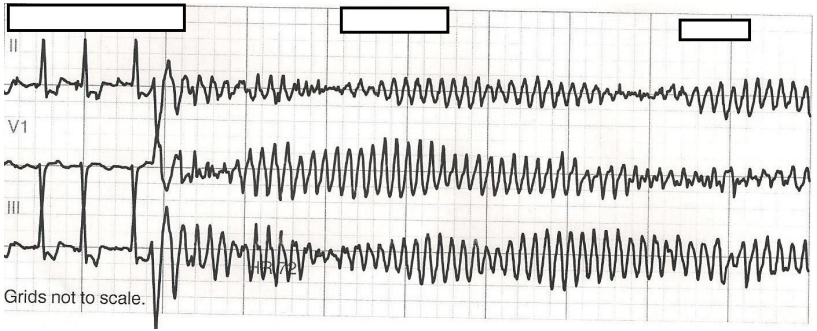
Hill coefficient (n) - ‘slope’ of the curve; define the ‘therapeutic window’

Some drugs can cause Torsades de Pointes (TdP)

Changes in cardiac function



ECG



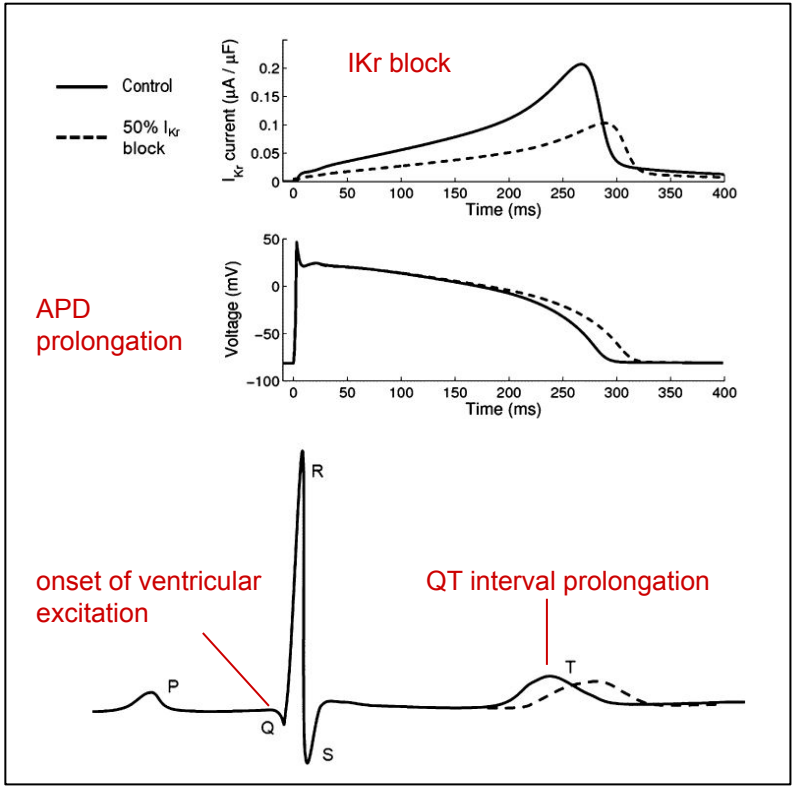
https://jetem.org/torsades_depointes/

→ Form of ventricular arrhythmia that can lead to sudden cardiac death.

→ Cardiac drug safety assessment guidelines require prediction of **TdP risk**.

→ TdP is the leading cause of drug-withdrawal from the market (Mirams et al., 2012).

hERG-channel block is used in drug safety studies as an indicator of pro-arrhythmic risk



Mirams et al., 2012

- ➔ The human ether-à-go-go related gene (hERG) channels in carry the 'rapidly activating delayed rectifier potassium' current ($\mathbf{I_{Kr}}$).
- ➔ Block of I_{Kr} prolongs AP duration (APD) and the QT interval.
- ➔ TdP risk has been associated with drug-induced QT prolongation.

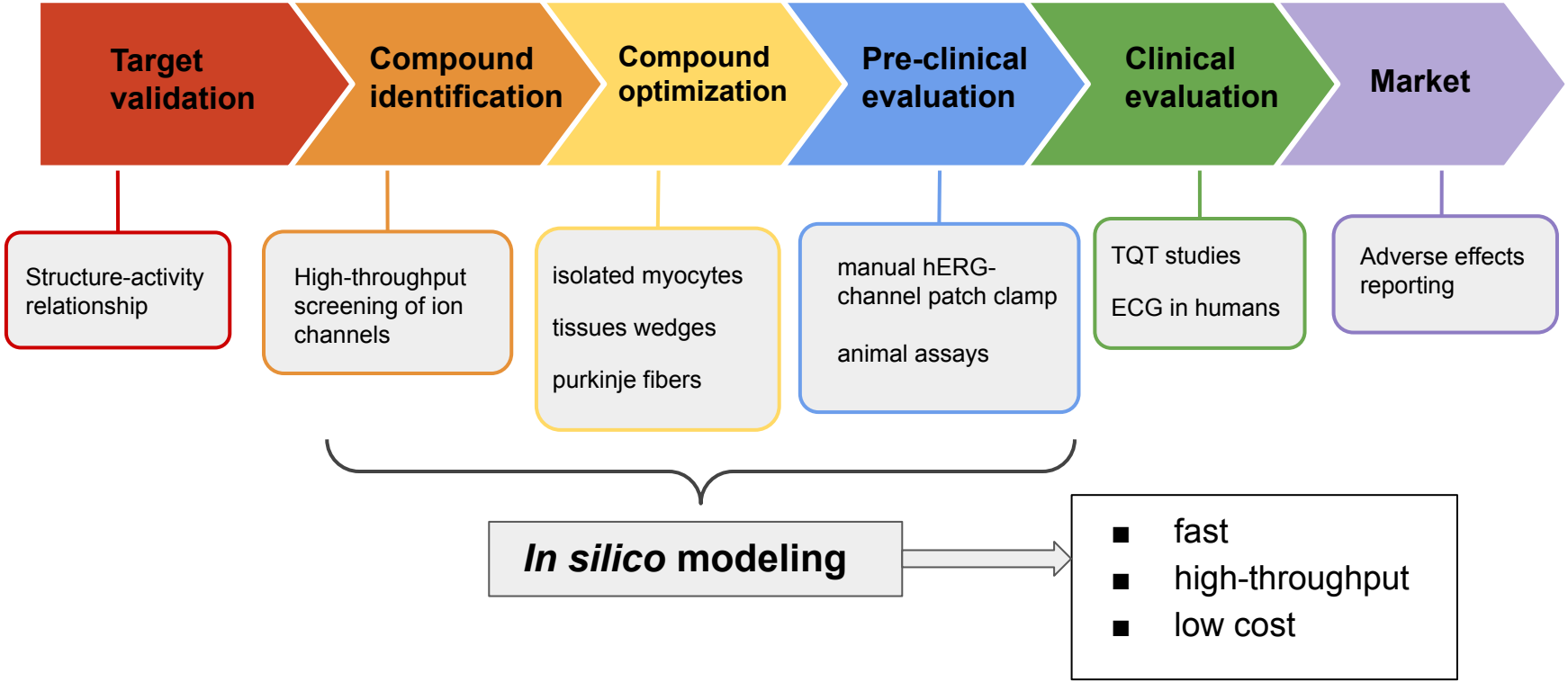
ICH-S7B

1. *In vitro* IKr patch clamp experiments using hERG expression systems.
2. *In vivo* QT measurements in conscious animals.

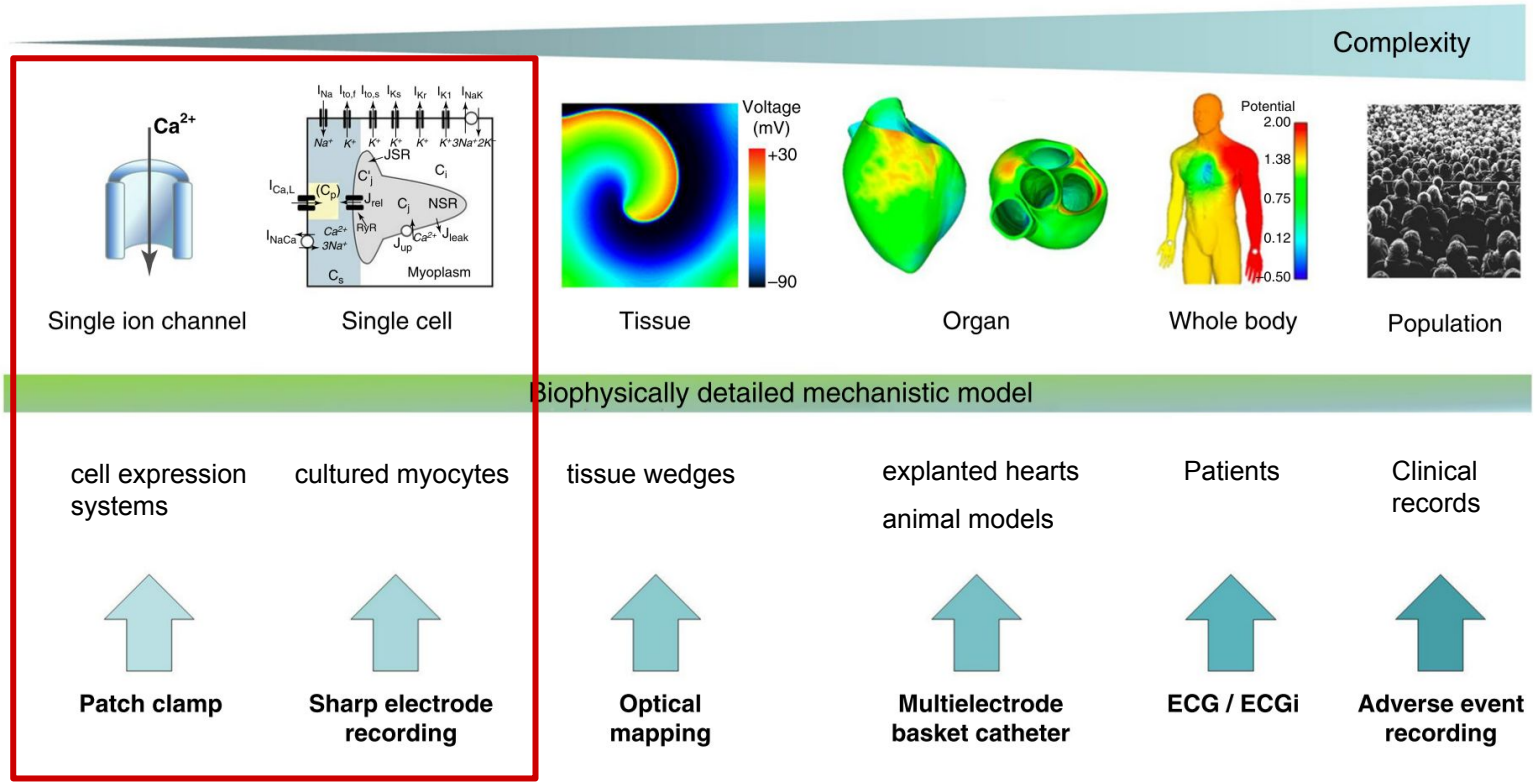
ICH-E14

3. Human phase II 'thorough QT' (TQT) study.

In silico approaches reduce the number of cell-based assays and animal tests needed, lowering development costs

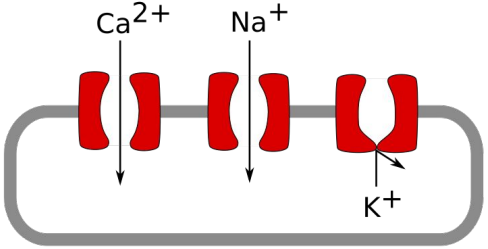


In silico approaches span all space and time scales, from ion channel to cardiomyocyte, to whole organ



Adapted from Davies et al., *Drug Discovery Today*, 21 (6), 2016

In silico models of cardiomyocytes are modeled as a system of differential equations



Transmembrane potential as function of ionic currents

$$\frac{dV_m}{dt} = -\frac{1}{C_m} I_{\text{tot}}$$

$$I_{\text{tot}} = \sum^{i} I_i + I_{\text{stim}}$$

Action potential

Hodgkin-Huxley models

$$I_i = g \cdot \prod^{j} x_j \cdot (V_m - E_S)$$

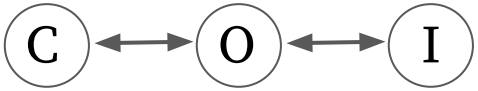
$$\frac{dx}{dt} = \alpha \cdot (1 - x) - \beta \cdot x$$

→ *g* is the **maximum conductance**

→ *x* are voltage- and time-dependent gating variables

→ α and β model activation and inactivation of gates

Markov models



$$I_i = g \cdot P(O) \cdot (V_m - E_S)$$

$$\frac{dP_i}{dt} = f\left(P_i(t), q(V_m)\right)$$

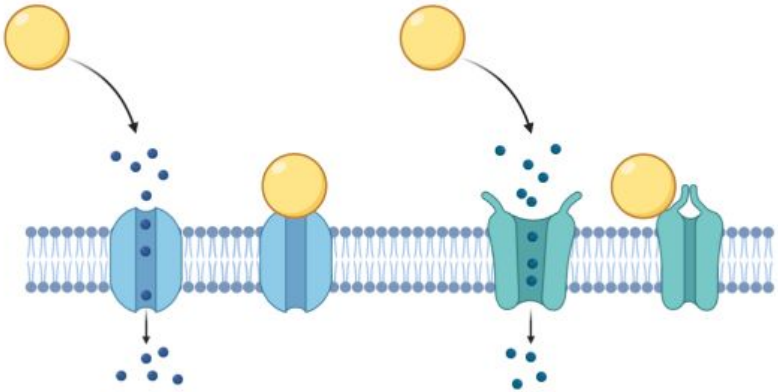
→ ion channel states explicitly modeled

→ voltage- and time-dependent state transition rates

Drug block is often modeled as direct binding of the drug to an ion channel

'*conductance-block*' (or pore) model

→ In some cases, a drug affects the ion-channel by direct binding



$$I_i = g \cdot P(O) \cdot (V_m - E_S)$$

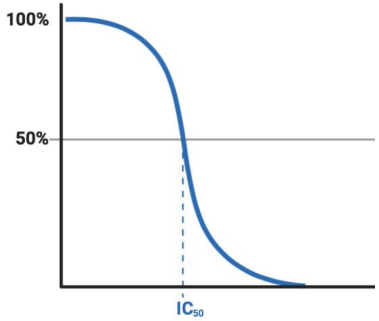
maximum conductance

Dose-response curve

→ maximum conductance of ion channel scaled by a factor *b* (proportion of blocking effect).

$$b = \frac{1}{1 + \left(\frac{[D]}{IC_{50}}\right)^n}$$

D is the compound dose

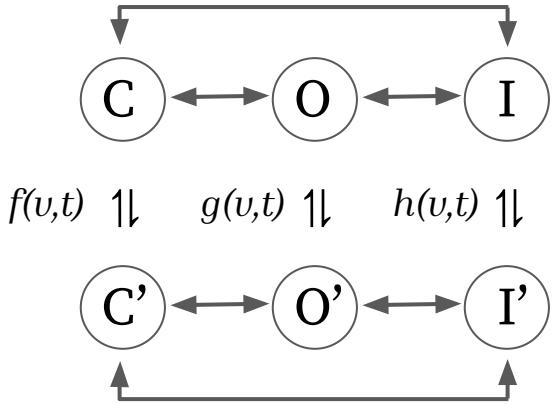


→ Voltage- and time- independent

Interactions between drugs and ion channels can be more complex, and often voltage- and time-dependent

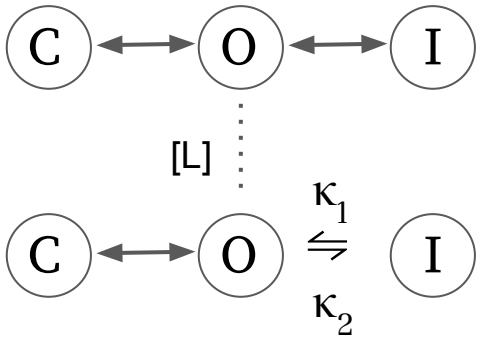
State-dependent block

drug binding creates new states with rates expressed as functions of voltage and time.



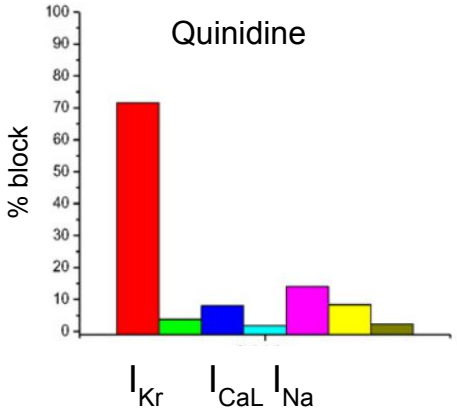
Allosteric block

bound drug alters the rates of transition between ion-channel states.

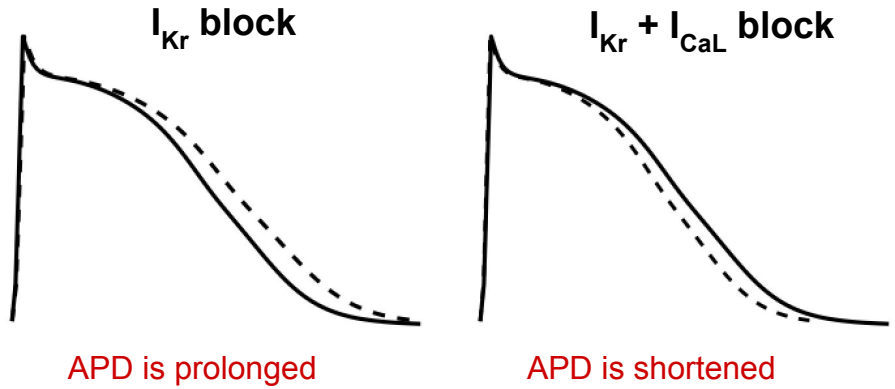


Most drugs block multiple channels, which affects model predictions

1. Drugs that block hERG may not cause TdP
2. Drugs that do not block hERG may cause TdP



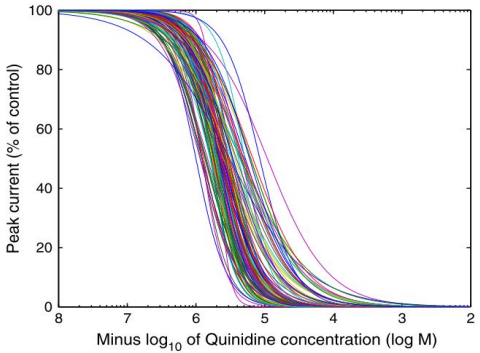
Adapted from Crumb et al., 2016



- block of different channels balance out effects on AP
- multi-channel block improves TdP risk
- Adopted by most pharmaceutical companies

Variability in experimental data introduces uncertainties in model predictions

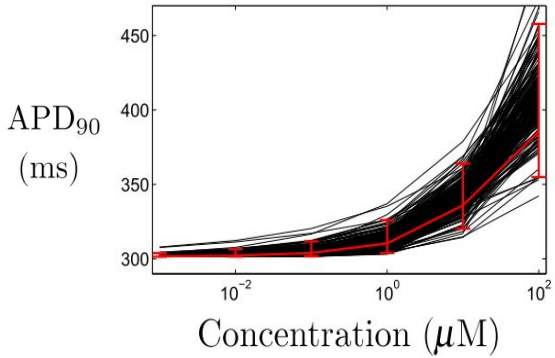
Variability in C-E curves



Elkins et al., 2013

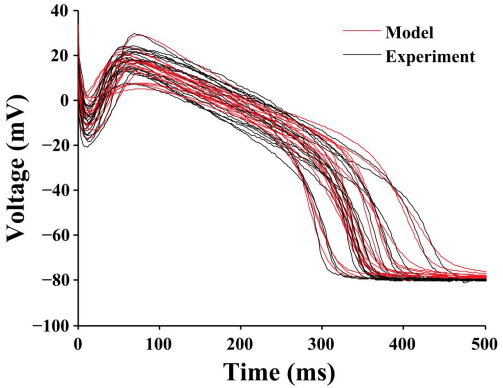
IC50, Hill coefficients?

Uncertainty in i*n silico* predictions



Elkins et al., 2013

Variability in AP traces



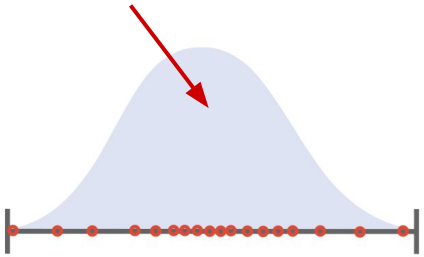
Davies et al. (2012)

Model parameters?

Experimental variability can be incorporated into *in silico* models to improve model predictions

Ion channel formulation

$$I_i = g \cdot P(O) \cdot (V_m - E_S)$$



Vector of maximum conductances

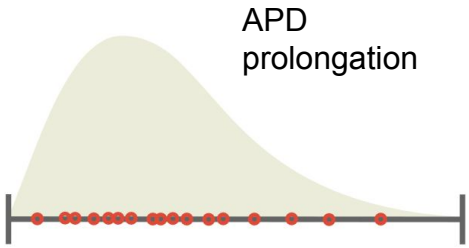
$$g = \left\{ g^{(1)}, g^{(2)}, ..., g^{(N)} \right\}$$

Population of model variants

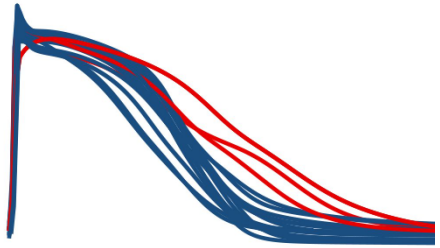
Pore block

$$b = \frac{1}{1 + \left(\frac{[D]}{IC_{50}} \right)^n}$$

Action potential properties



Drug susceptibility

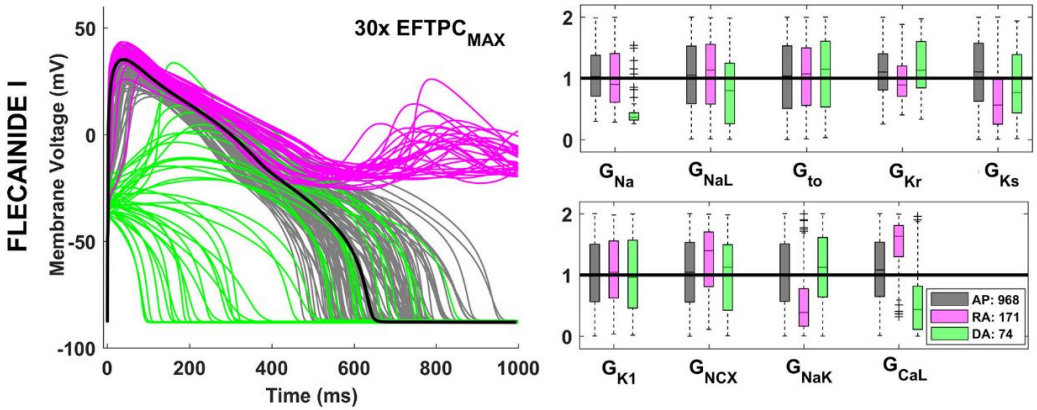


Populations of human ventricular CM models can predict risk of drug-induced arrhythmias more accurately than animal models

→ Passini et al. (2017) incorporated **inter-subject** variability into pro-arrhythmia prediction models

→ High predictability of TdP risk

→ More accurate predictions of drug effects on subpopulations



Normal
Repolarization abnormalities
Depolarization abnormalities

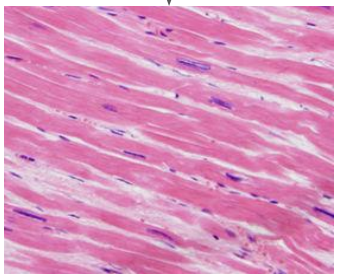
EFTPC$_{max}$: maximal effective free therapeutic concentration

62 compounds (all TdP risk categories)

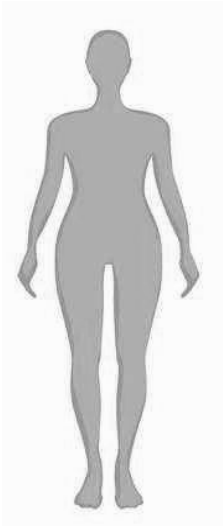
based on RA		based on ΔAPD_{90}	
TRUE + 32	TRUE – 23	TRUE + 34	TRUE – 16
FALSE + 2	FALSE – 5	FALSE + 9	FALSE – 3
Sensitivity: 87%		Sensitivity: 92%	
Specificity: 92%		Specificity: 64%	
Accuracy: 89%		Accuracy: 81%	

Passini et al., Frontiers in Physiology 8, 2017

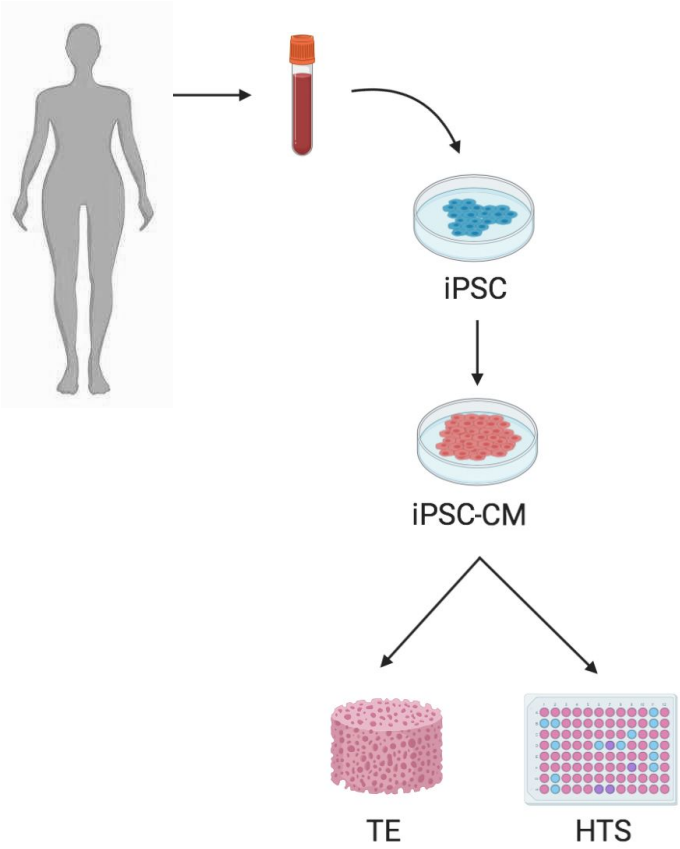
Drug effect predictions from animal assays not always produce accurate predictions on humans



Drug safety predictions



iPSC-CMs are a novel and promising *in vitro* model of human cardiomyocytes



Induced pluripotent stem cell-derived cardiomyocytes (iPSC-CMs)

→ *In vitro* model for high-throughput drug safety tests

→ Readily obtained and renewable source of human CMs

→ Patient- and disease-specific

Limitations

→ Immature phenotype

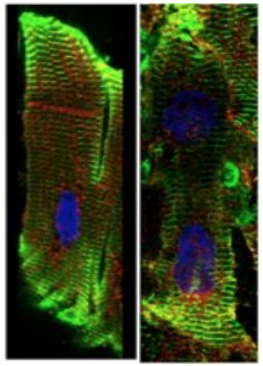
→ *In vitro* characterization

→ Analytical methods to translate predictions from iPSC-CMs to human ventricular CMs (hV-CM)

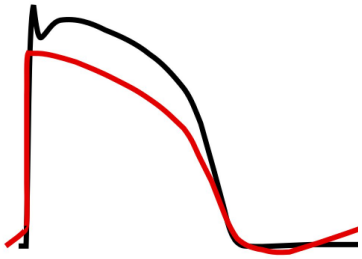
Differences between iPSC-CMs and adult cardiomyocytes require dedicated *in silico* models

- different cell morphologies
- different expression levels of ion channels
- spontaneous electrical activity

hVCM iPSC-CM



Lemoine *et al.Sci Rep* 7, 5464 (2017).

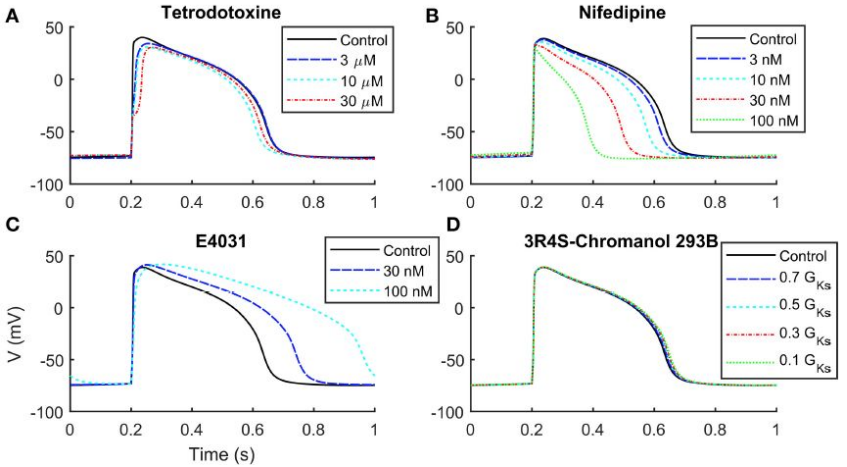


iPSC-CM AP

hV-CM

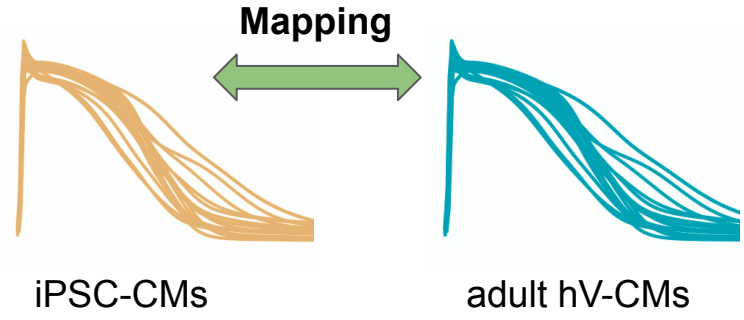
Paci model

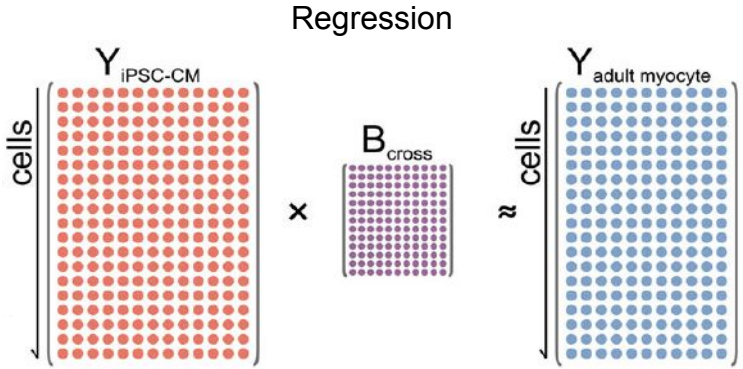
→ extensively validated against experimental data



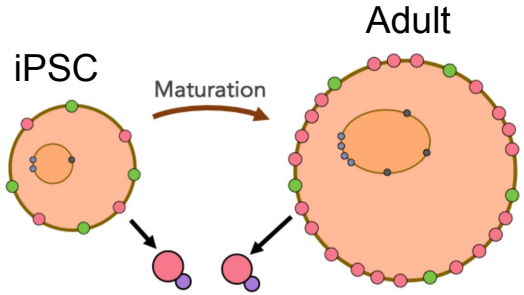
Paci et al., 2018

How to translate drug effects on iPSC-CMs onto adult cells?





Gong et al., 2018



Adapted from Jæger et al., 2020

Maturation matrix (Q) of scaling factors of *g*

- data assimilation from optical voltage and Ca^{2+} measurement

7. CiPA initiative

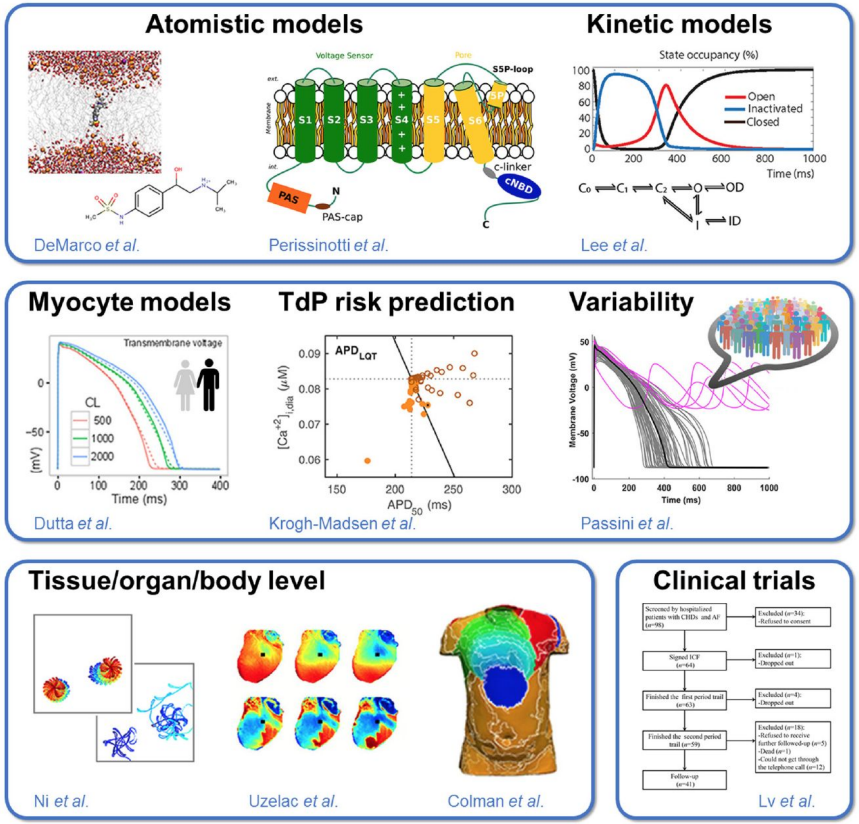
Develop and validate a new paradigm for pro-arrhythmic evaluation of new drugs

More accurate and integrative:

1. HTS of drug effects on multiple ion channels;
2. in silico modelling of human CM to assess electrophysiological responses;
3. verification of responses in iPSC-CMs

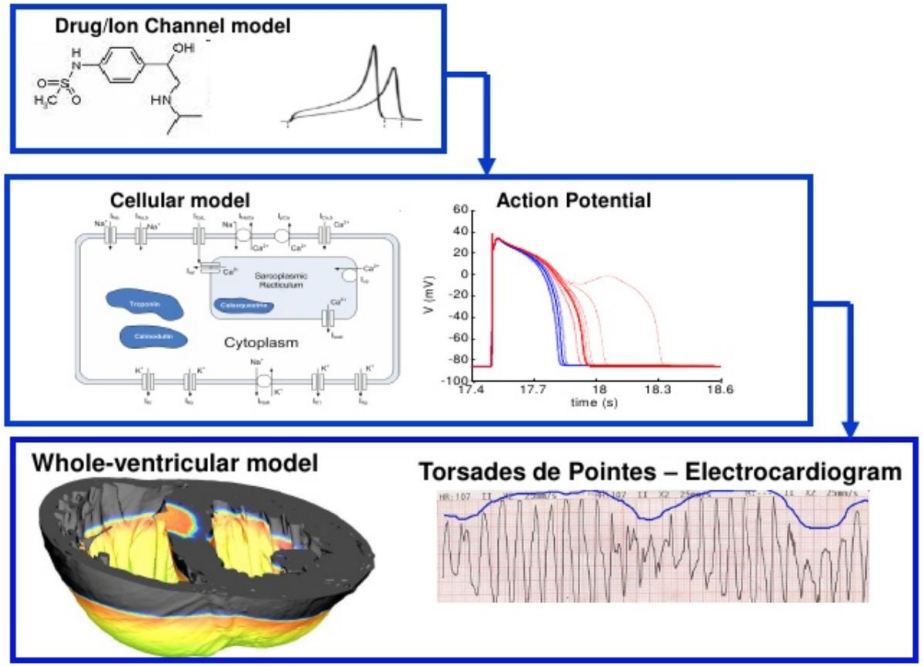
Different models predict different drug responses:

→ Proposes that each model is evaluated against a set of 28 compounds with variable proarrhythmic potential



Grandi et al., Frontiers in Physiology 9, 2018

8. Conclusions



Marco Viceconti, WoHIT 2010

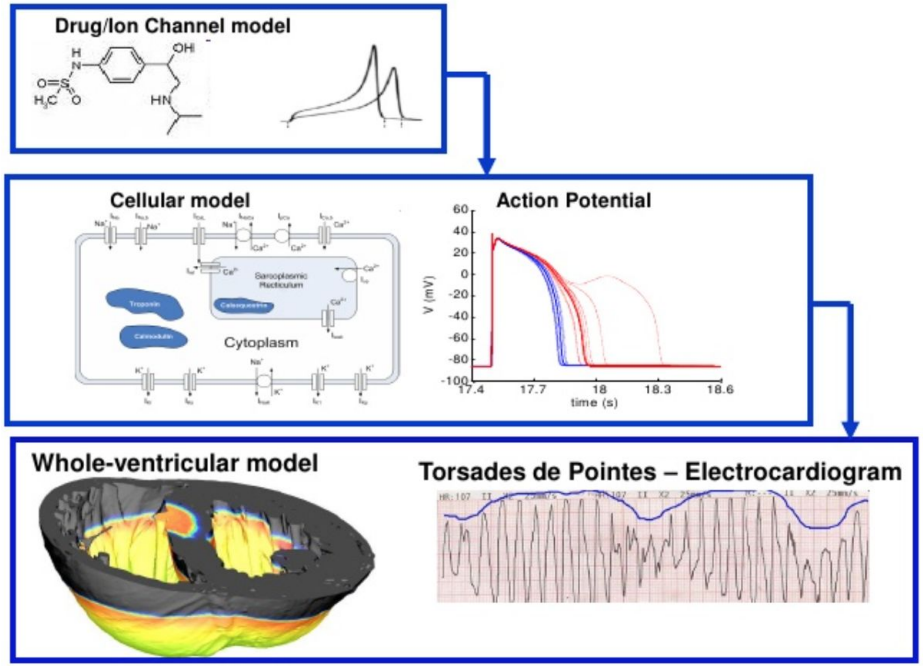
Limitations

1. Models cannot predict novel off-target drug effects if these are not represented in the model (eg., up- or down-regulation of ion channel expression);

2. The validity of results depend on the accuracy of the models;

3. Limited representation of drug interaction mechanisms to allow systematic, accurate, and high-throughput prediction of drug actions on the heart.

8. Conclusions



Marco Viceconti, WoHIT 2010

Future directions

1. Improve cell models to incorporate additional cellular mechanisms that may be affected by drug interactions.

2. Improve whole heart models by including the atria, the cardiovascular system, non-excitable tissues, mechano-electrical coupling, regional heterogeneity, etc.

3. Patient-specific phenomics, such as cardiac electrophysiology, disease states, genetic conditions, autonomic control, energy use, etc.

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