Mechanistic Systems Biology Modeling Applied to the Pre-Clinical Cardiac Safety Assessment of a Pharmaceutical Compound:

from Channels to Cells to Tissue

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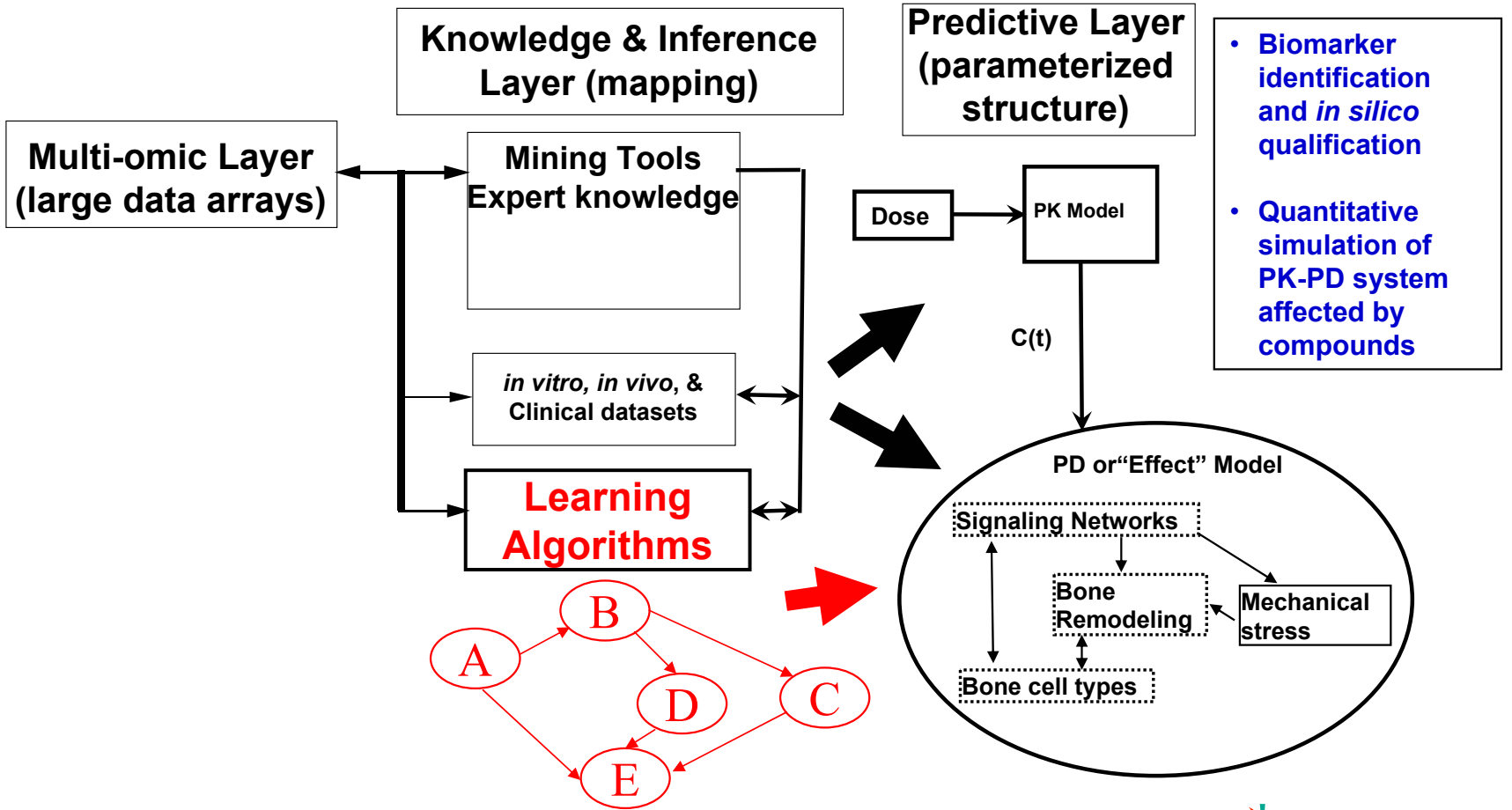
An Integrative Modeling Process



- Biomarker identification and *in silico* qualification
- Quantitative simulation of PK-PD system affected by compounds

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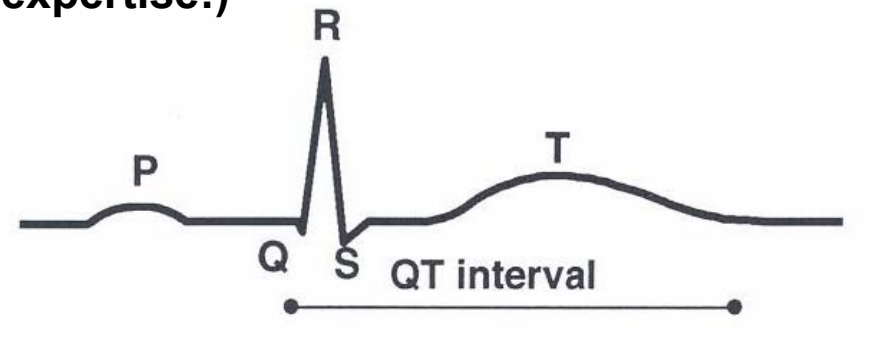
Cardiac Safety Assessment of Compounds

Potential for QT prolongation?

- One of the main reasons to withhold approval and withdraw drugs from the market
- Is associated with an enhanced risk for a specific ventricular arrythmia, which may lead to tachycardia (TdP), which may lead to death
- Very complex issue: many unknowns, multiple opinions, multiple risk factors, other important readouts for arrythmogenicity (TDR), integrated assessment of multiple endpoints to evaluate clinical risk (+range of expertise!)



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Pre-Clinical Cardiac Safety Assessment: Work flow on the Experimental Side

CHANNEL ASSAYS
(IC_{50} values)

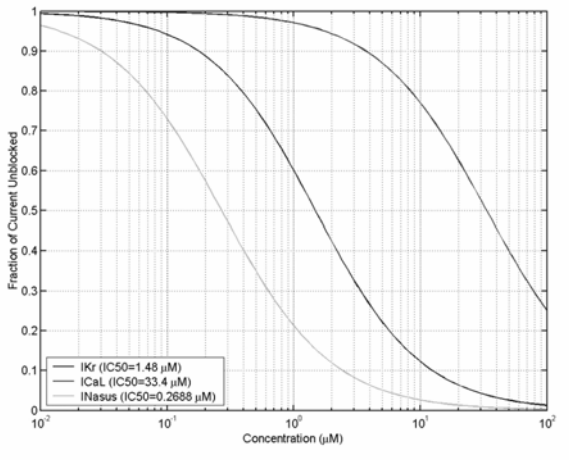
- **HERG (I_{Kr})**
- **Others (I_{Na})**

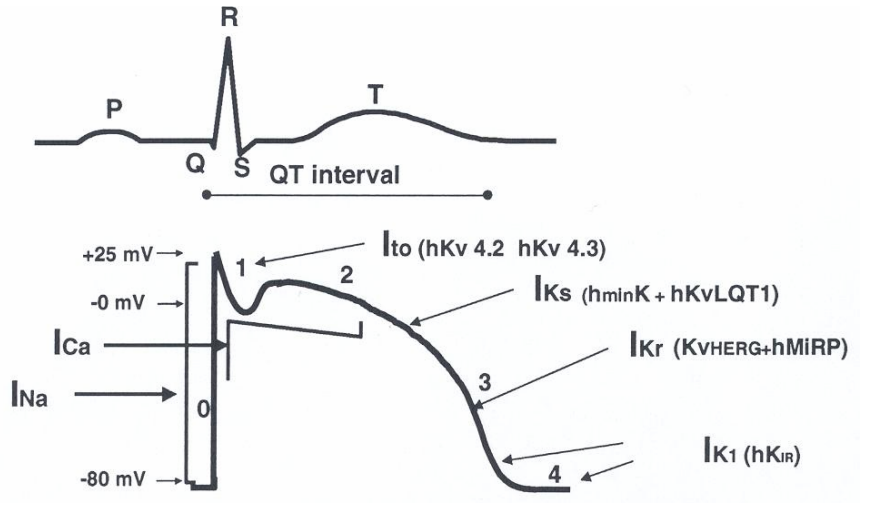
CARDIAC CELL/TISSUE ASSAYS
(Action Potential data)

- **Ventricular myocytes (various species, incl. human)**
- **Purkinje fiber (various species, incl. rabbit, canine)**
- **Papillary muscle, ventricular strips**

ORGAN LEVEL ASSAYS
(Higher level data, incl. ECG)

- **Langendorff type preparations, isolated rabbit heart**
- **In vivo dog telemetry**





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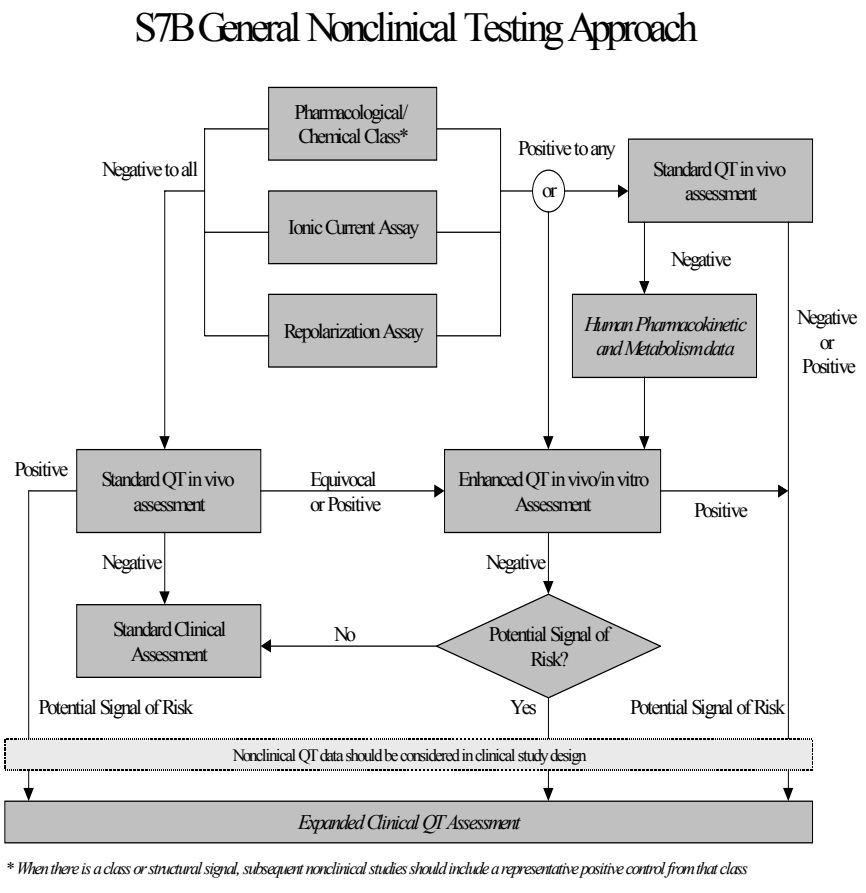
Tiered Risk Assessment



CARDIAC SAFETY INDICES

- **CSI = IC_{50u} / EC_{50d}**
- **CSI = IC_{50u} / IC_{50d}**
- **CSI = IC_{50u} / $[Plasma]_{free}$**
- **CSI = IC_{50u} / [Myocardium]**
- **Acceptable value**

= F[therapeutic class, benefit/risk factor, corporate policy, regulatory guidance]

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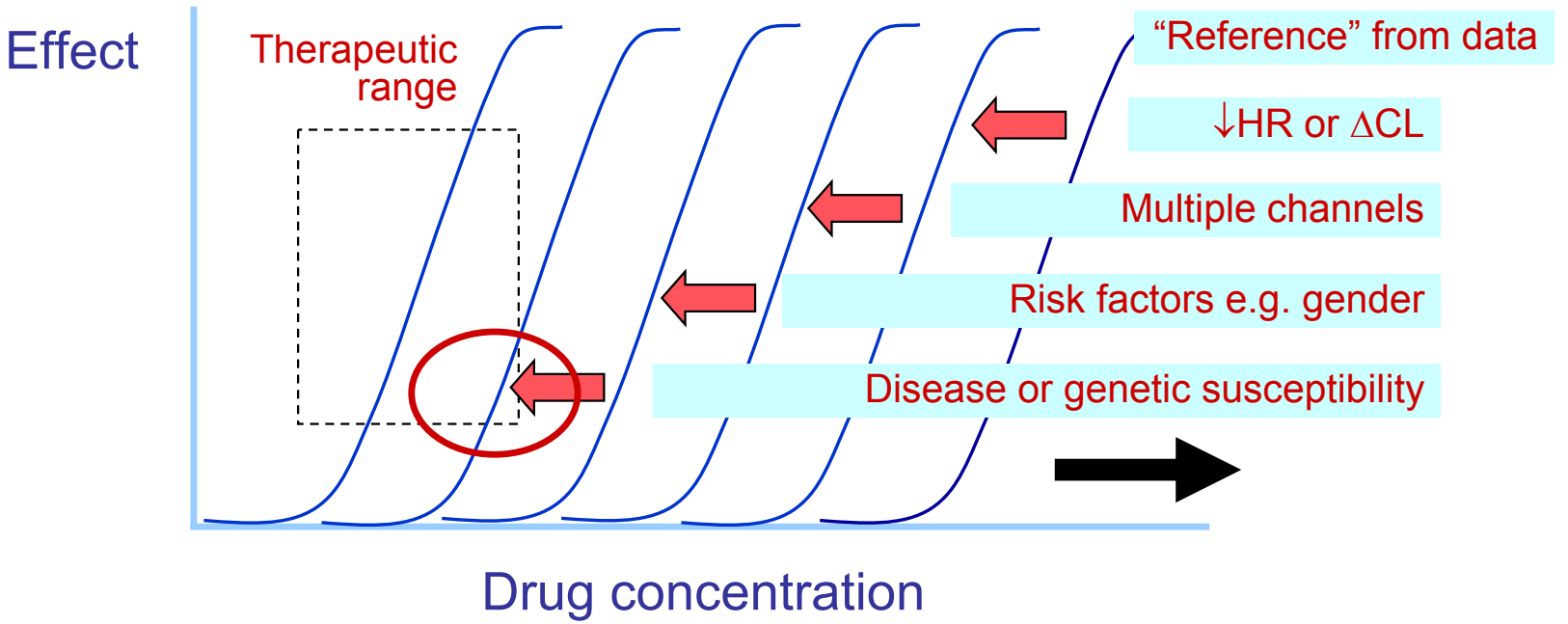
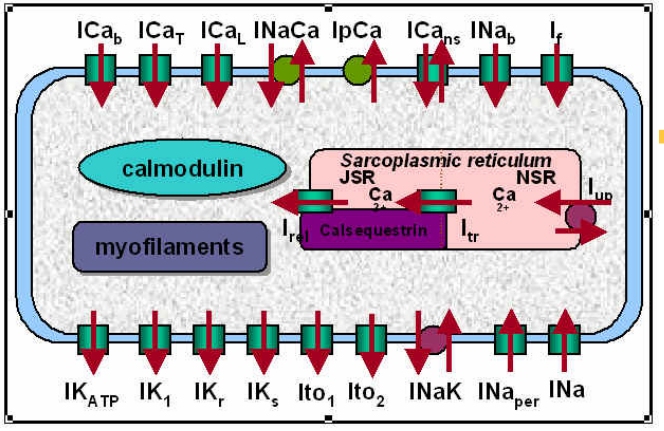
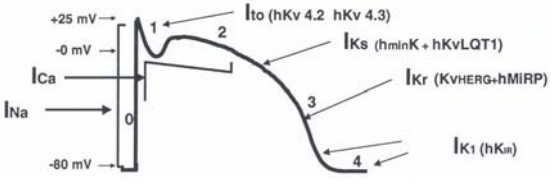
Safety Margins

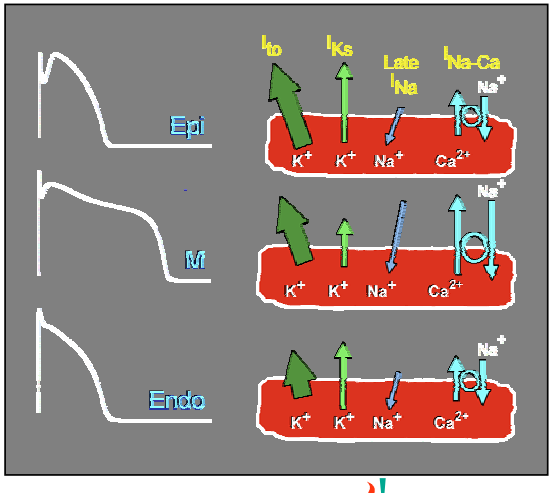


Integrating Channel Responses

- I_{Kr}: often the only channel directly tested at early screening stage
- Drugs often affect other channels: I_{Ks}, $I_{Ca\text{-}L}$, late $I_{na\text{-}sus}$, all important in repolarization!
- I_{Kr} "red flag signal" ➔ Mixed effects on other channels may worsen OR improve effects on APD and QT
- <u>NO</u> I_{Kr} "signal" ➔ Doesn't imply one is necessarily "safe" at the APD or QT level!
- Spatial heterogeneity in channels, from endo- to mid- to epi-cardiac cells across ventricular wall
- Many other physiological variables ➔ heart rate, disease/genetic status, gender, nutrition, diurnal







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CardioPrism™ (Physiome – Novartis, 2002)

CHANNEL DATA
(IC_{50} values)

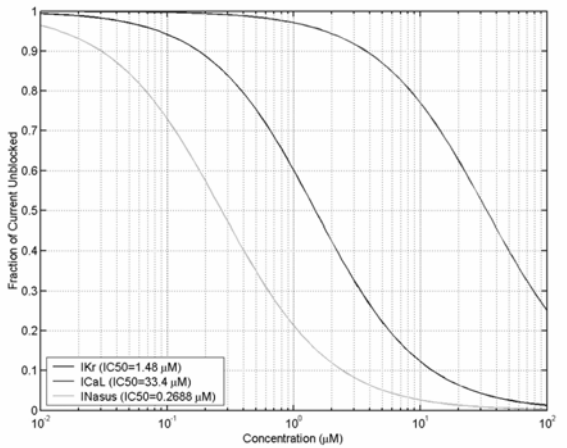
- **HERG (I_{Kr})**
- **Others (I_{Ca-L}, I_{Na-sus}, I_{Na-Ca}, I_{Ks}, I_{to},)**

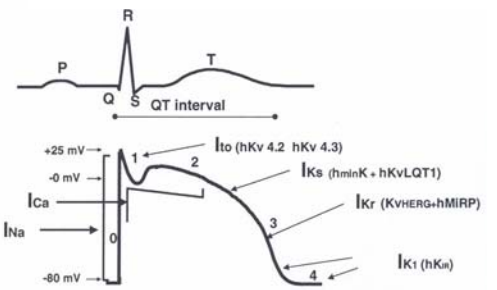
CARDIAC CELL MODELS
- **GP ventricular cell**
- **Canine ventricular cell (epi, endo, mid)**
- **Human ventricular myocyte**
- **Rabbit Purkinje fiber**

Action Potential
(& its characteristics: APD, etc)

TISSUE MODELS
- **Canine "tissue cable" model**

Integrated transmural signal, "ECG-like" (with characteristics such as QT interval, TDR, etc)





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CardioPrism™ Platform: Basis for Integrated Models

Suite of cardiac cell models for multiple species built upon:

- **J.J. Rice (+ Ca^{2+} handling), M.S. Jafri, R.L. Winslow**
- **D. Noble *et al*.**
- **C. Antzelevitch *et al*.**

Spatial aspects & integration at tissue level (modeling & experiments):

- **P.J. Hunter *et al*., Y. Rudy *et al*. (1-D "cable" model)**
- **C. Antzelevitch *et al*.**
- **Physiome: A. Muzikant, C. Penland, G. Chen (based on previous work, Duke U.)**

Compound entry: **model assumes that the channel-specific ($IC_{50,x}$) and Hill Coefficient (*Nx*) characterize a sigmoidal dose-response relationship for the inhibition of current (*Ix* vs. *Ix,control*) as a function of [Drug]**

$$\frac{I_x([Drug])}{I_{x,control}} = \frac{1}{1+\left(\frac{[Drug]}{IC_{50,x}}\right)^{N_x}}$$

CardioPrism™: Drug A *vs.* Drug B

CHANNEL DATA
(IC_{50} values)

- **HERG (I_{Kr})**
- **Others (I_{Na}, I_{Ca})**

CARDIAC CELL MODELS
- **GP ventricular cell**
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- **Human ventricular myocyte**
- **Rabbit Purkinje fiber**

- **Canine Purkinje fiber**

Action Potential
(& its characteristics: APD, etc)

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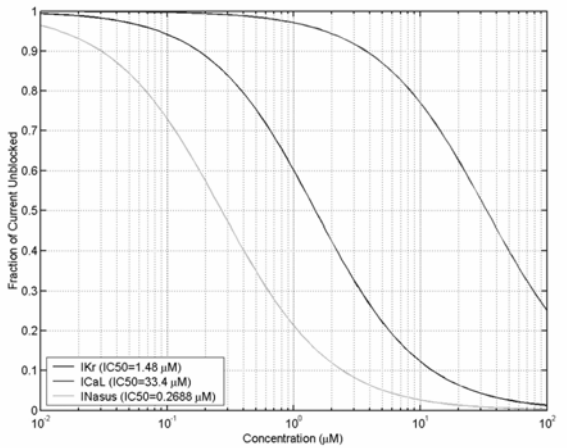

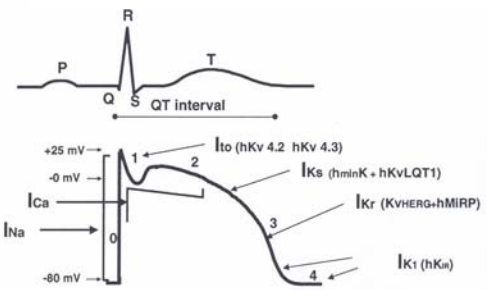

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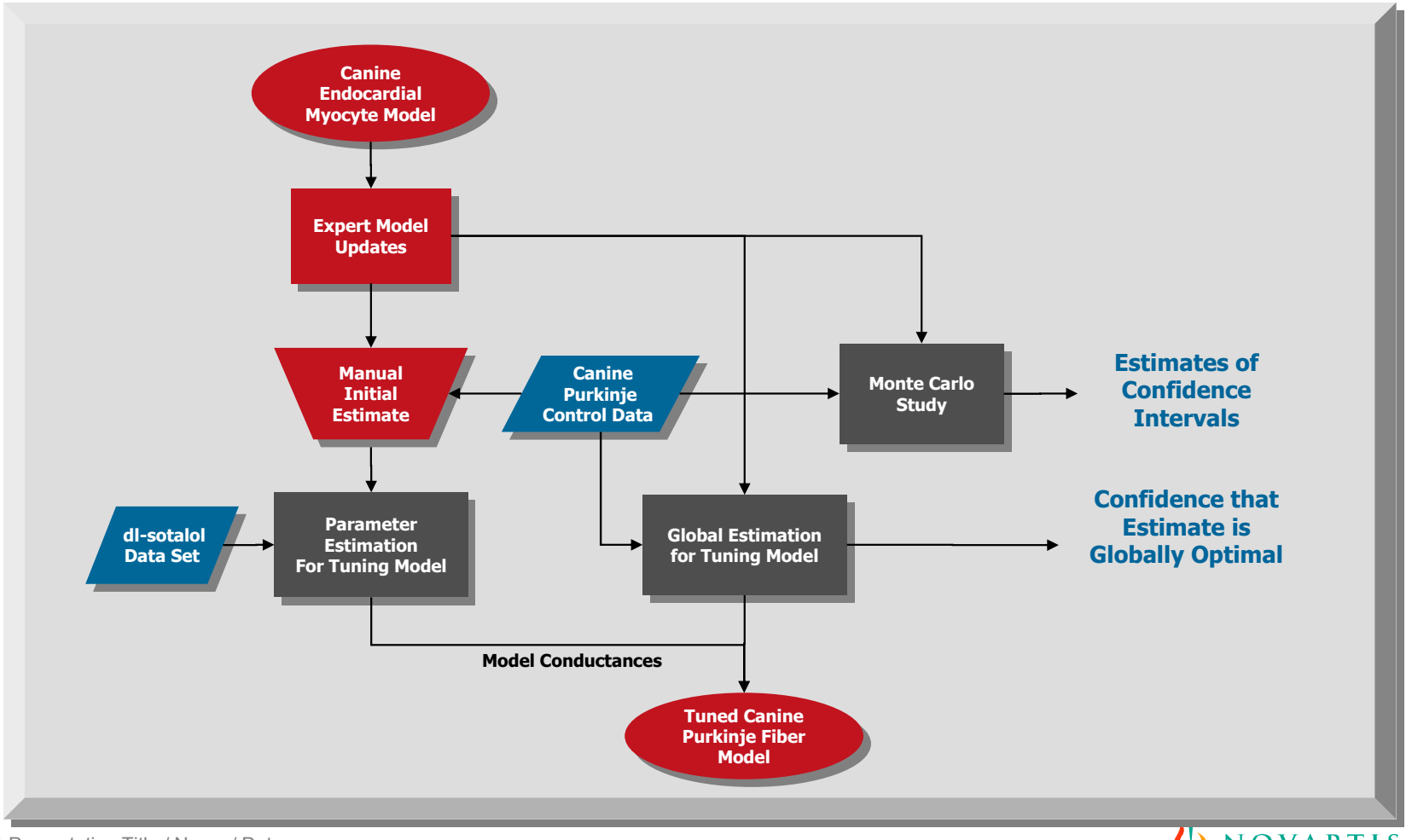
CardioPrism™: (I) Development of New Canine Purkinje Fiber Model



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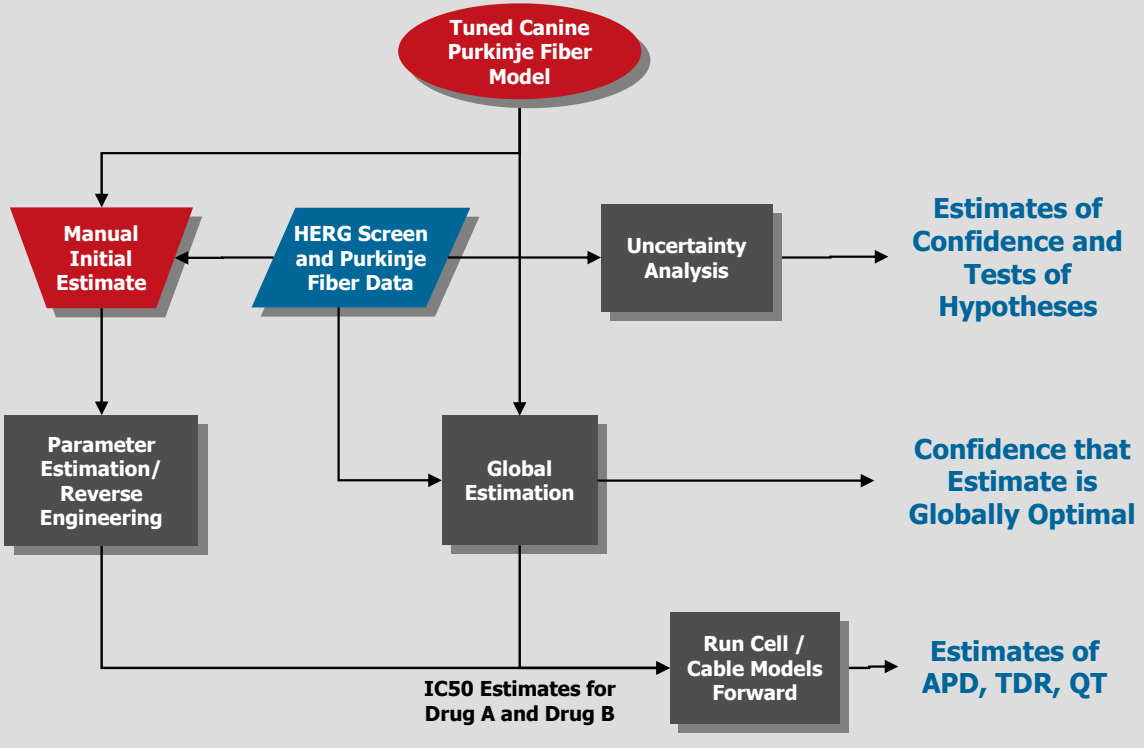
CardioPrism™: (I) Development of Canine Purkinje Fiber Model: Assumptions

- **Mechanisms within the endocardial myocyte model are similar to those of canine Purkinje fiber**
  - **Differences can be approximated by changing 14 conductance parameters**
- **Drugs act via a sigmoidal dose-response relationship to inhibit 6 currents (I_{Kr}, I_{Ks}, I_{to}, $I_{Ca\text{-}L}$, $I_{Na\text{-}Ca}$, $I_{Na\text{-}sus}$)**
  - **These currents suffice to predict the action of a drug on ventricular myocytes and Purkinje fibers**
- **Dose-response parameters from HERG assay and Purkinje fiber parameter estimates can be used in ventricular myocyte models**
- **The chosen error functions are a good measure of the quality of fit of the model to action potential data**

CardioPrism™: (II) Reverse-, then Forward-Engineering



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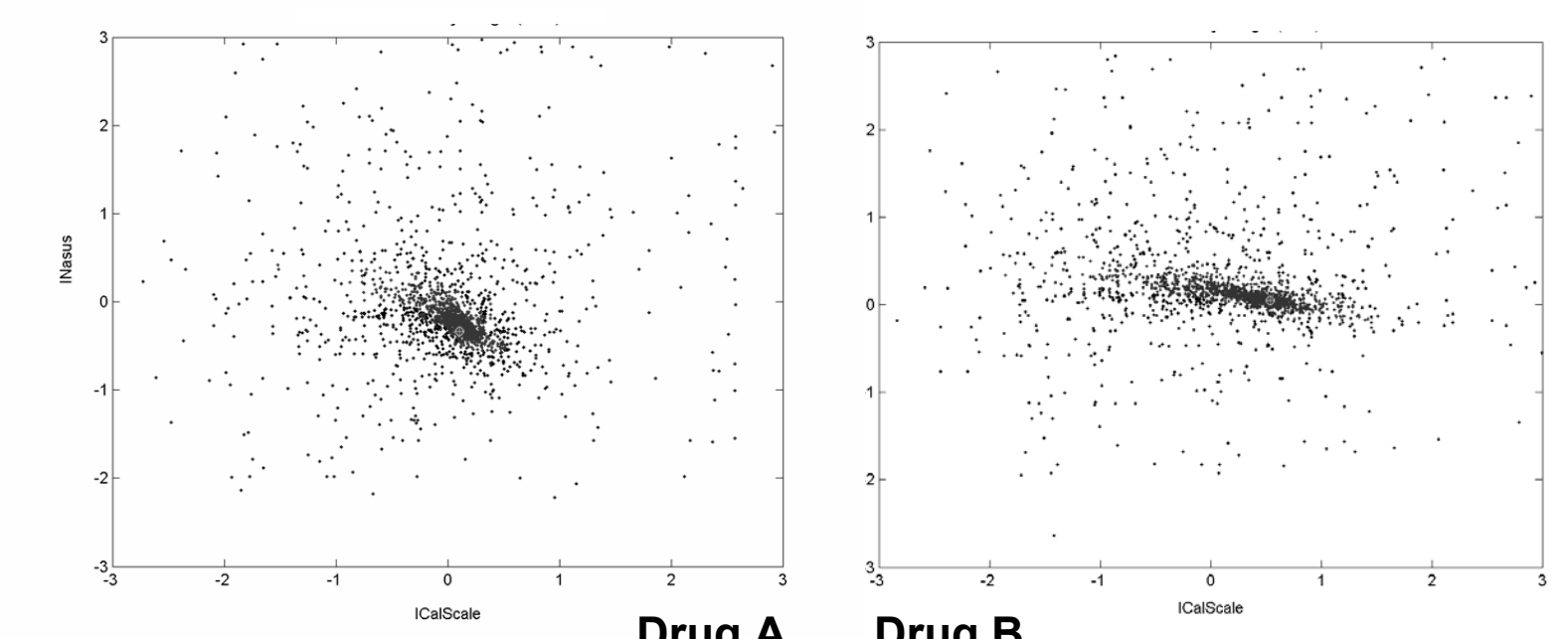
CardioPrism™: (II) Reverse-Engineering: Global Estimation Results



Drug A **Drug B**

- **Target shows location of best IC50 fits for $I_{Na\text{-}sus}$ & $I_{Ca\text{-}L}$**
- **Clustered points are next best fits (simulations with errors less than twice the error of the best fit)**

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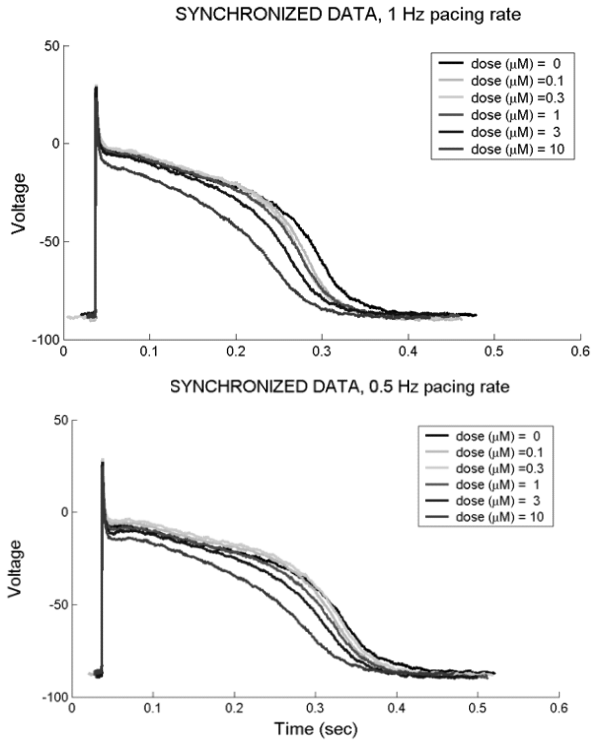
Data Set: Drug A vs. Drug B
HERG current is inhibited, but APs are not prolonged

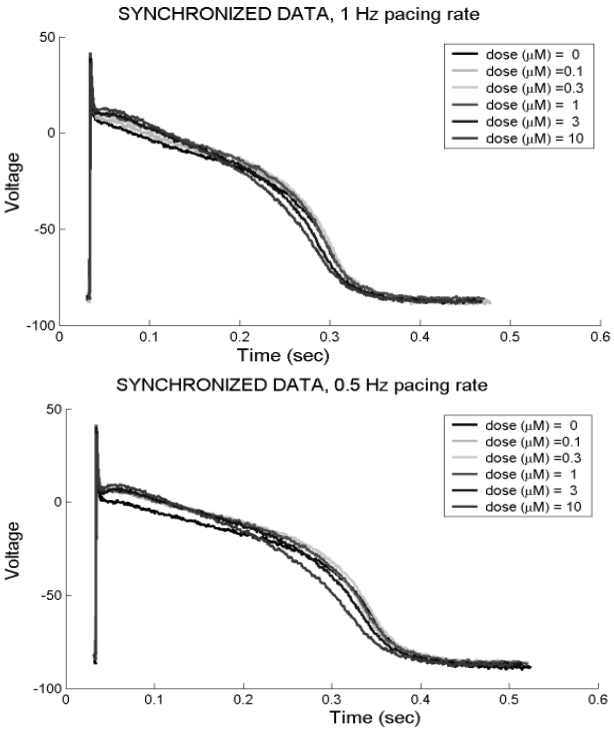
IC_{50} for I_{Kr} ($hERG_{HEK293}$)

- 1.48 μM (Drug A)
- 1.82 μM (Drug B)

Isolated canine Purkinje fiber

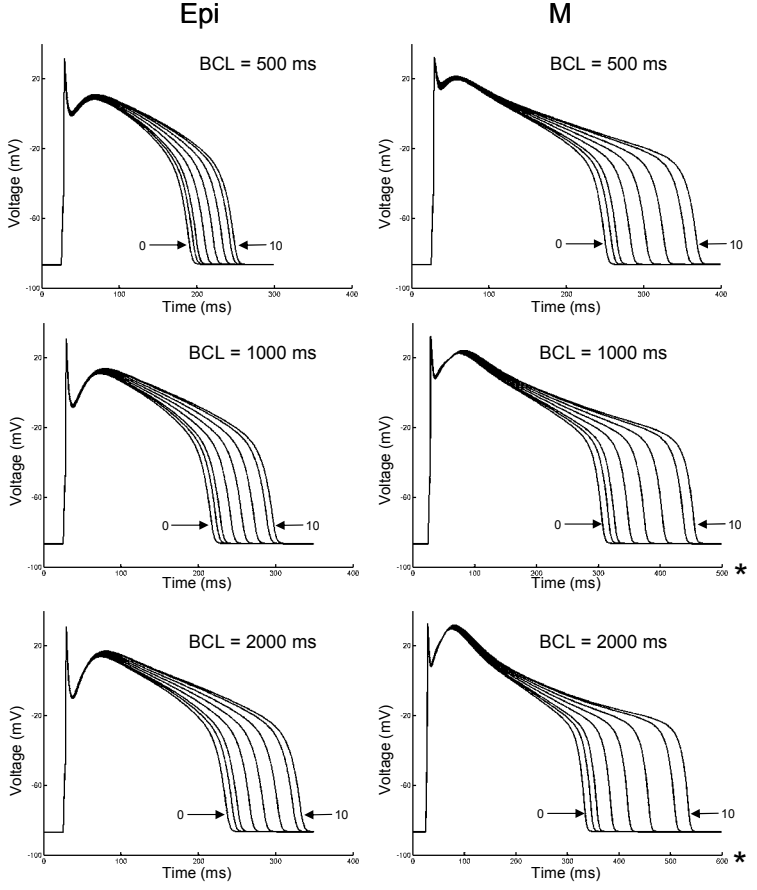
- paced at 0.5, 1.0 Hz,
- 0.0, 0.1, 0.3, 1.0, 3.0, 10 μM



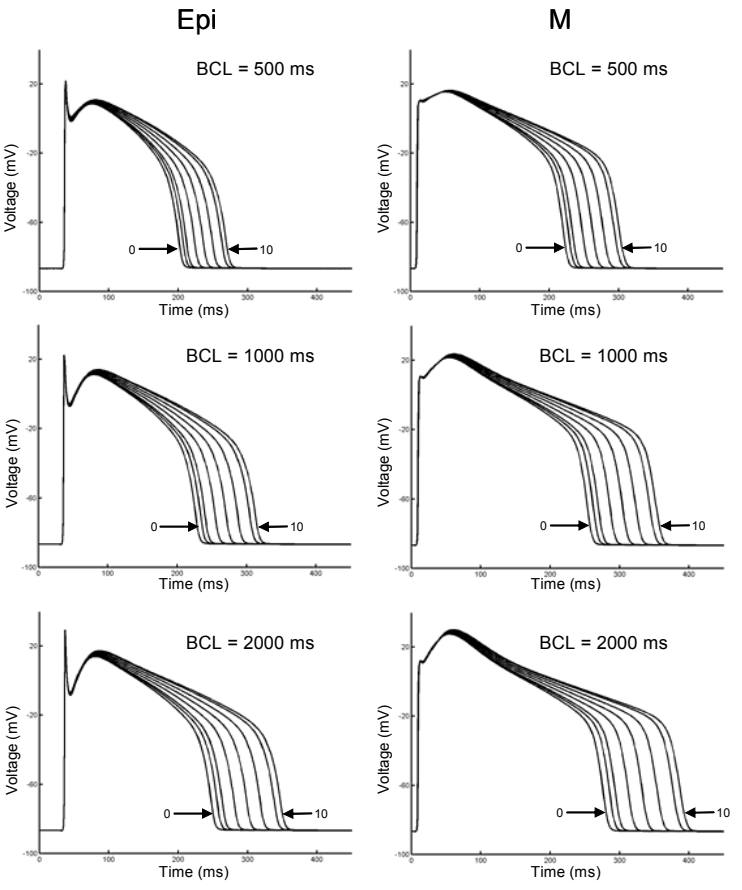


CardioPrism™: "Pure I_{Kr} Blocker" Hypothesis (Drug A & B) ➔ Not good, but we knew this

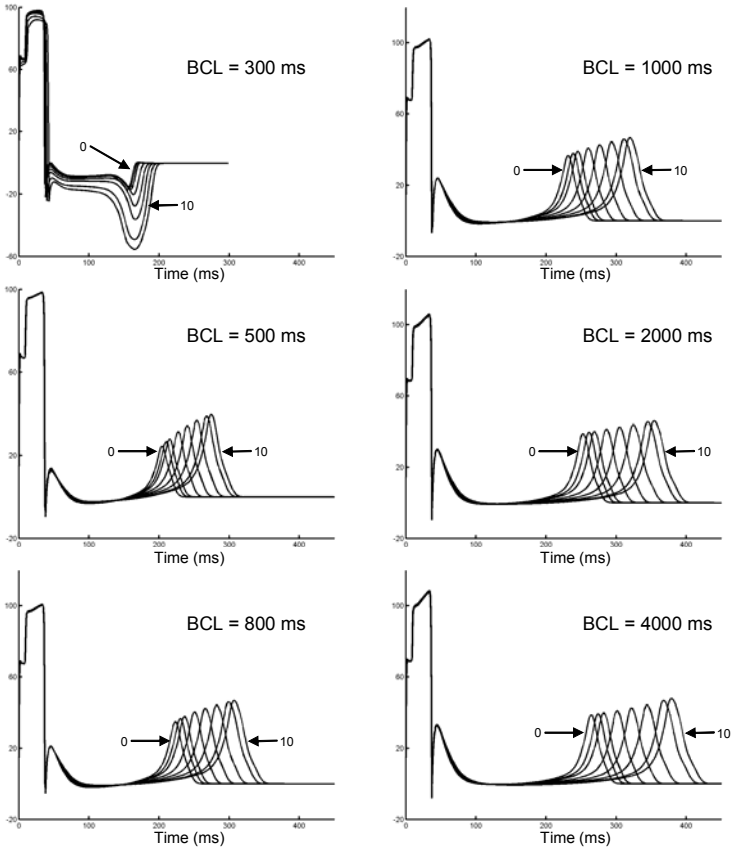
Isolated Cell Models

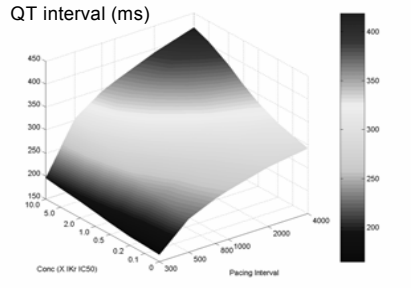


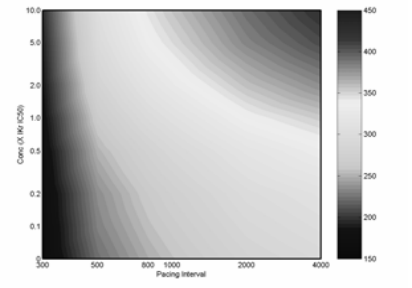
Cells Coupled Through the Cable Model

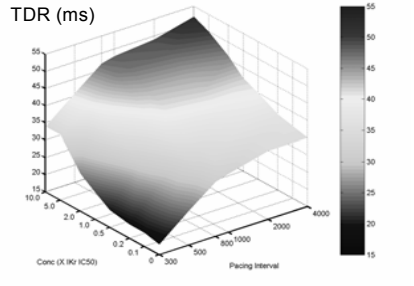


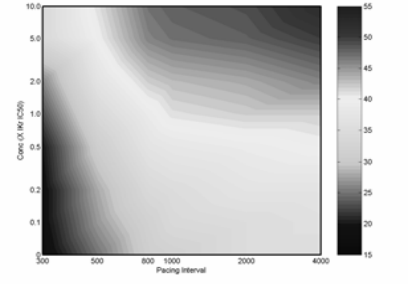
CardioPrism™: "Pure I_{Kr} Blocker" Hypothesis (Drug A & B)

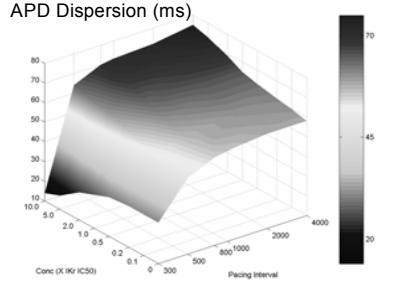


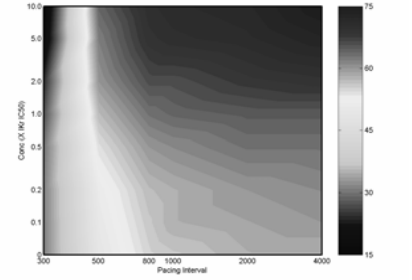












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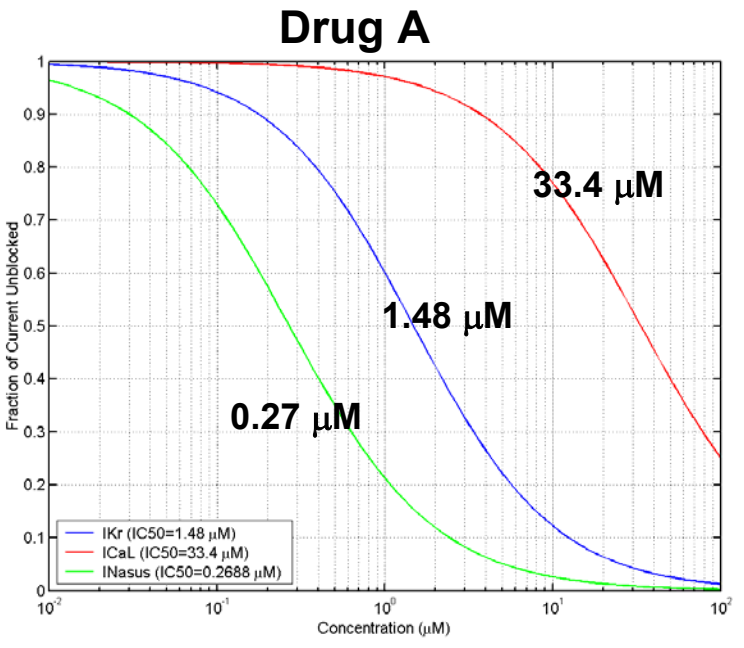
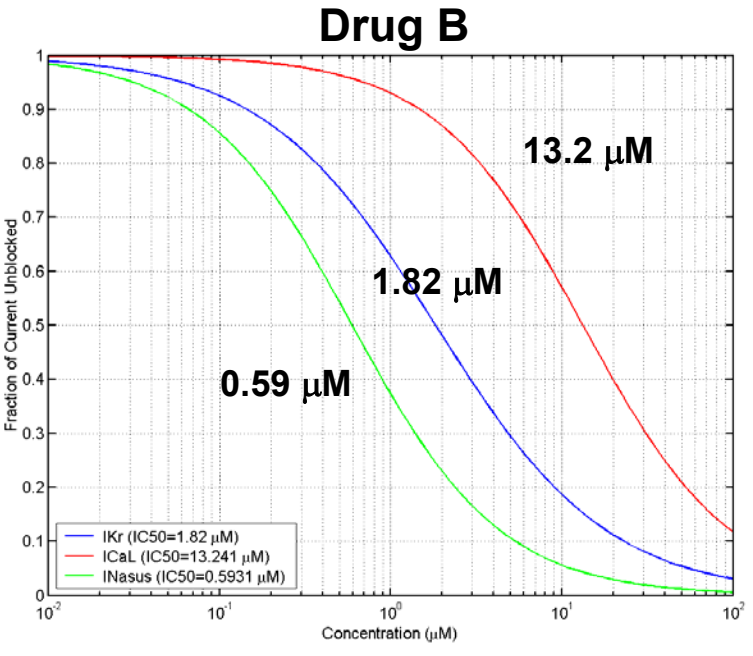
CardioPrism™: Reverse-Engineering & Prediction of IC_{50}'s for late $I_{Na\text{-}sus}$ and $I_{Ca\text{-}L}$ Currents

➔ Significant inhibition of $I_{Na\text{-}sus}$, I_{Kr}, $I_{Ca\text{-}L}$ by both drugs
➔ Dose-response estimates for key currents: important for AP repolarization





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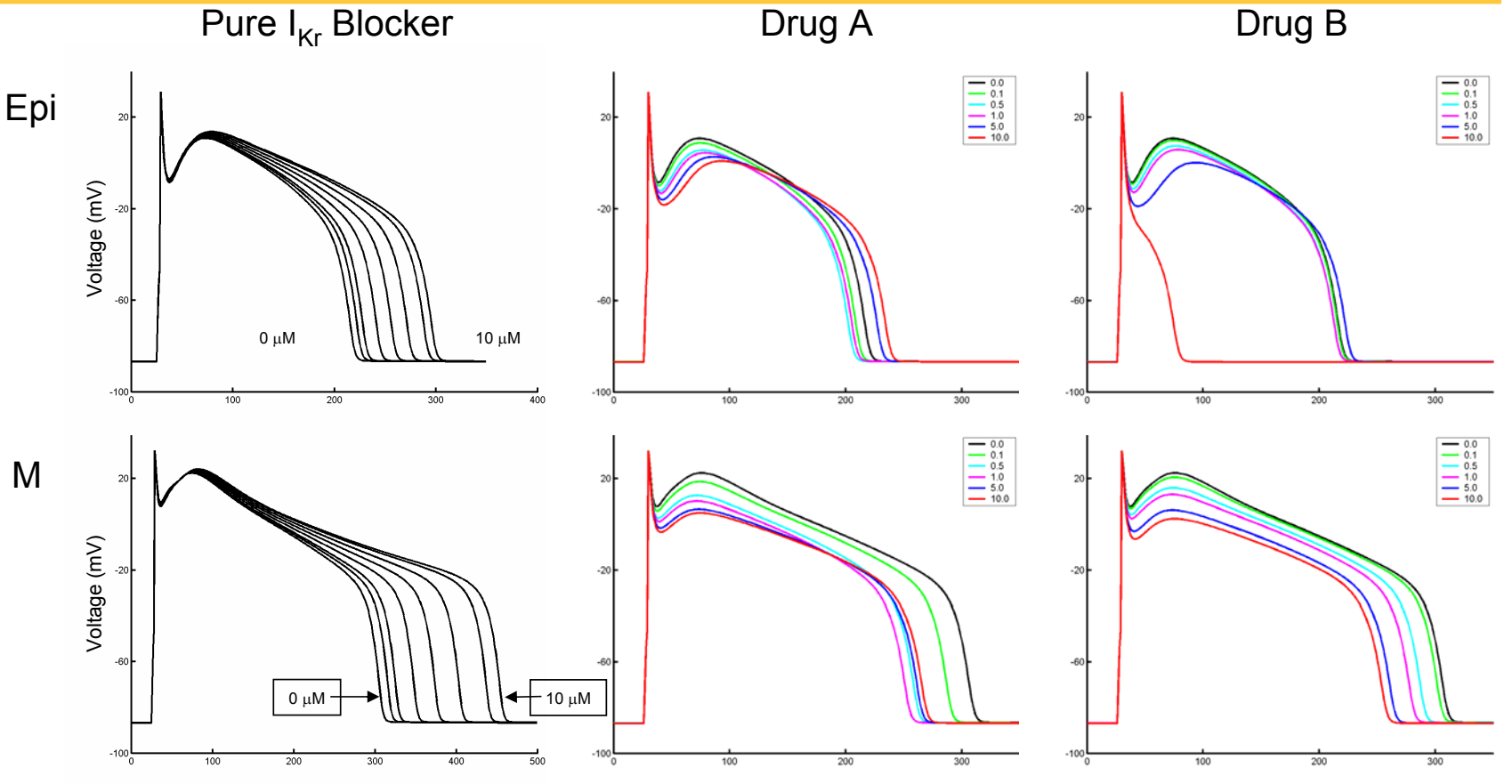
CardioPrism™: Forward-Engineering (I)



In contrast to pure I_{Kr} blockers, which prolong the action potential (severely so in M cells), Drug A & Drug B either do not affect or even shorten action potentials in isolated cells

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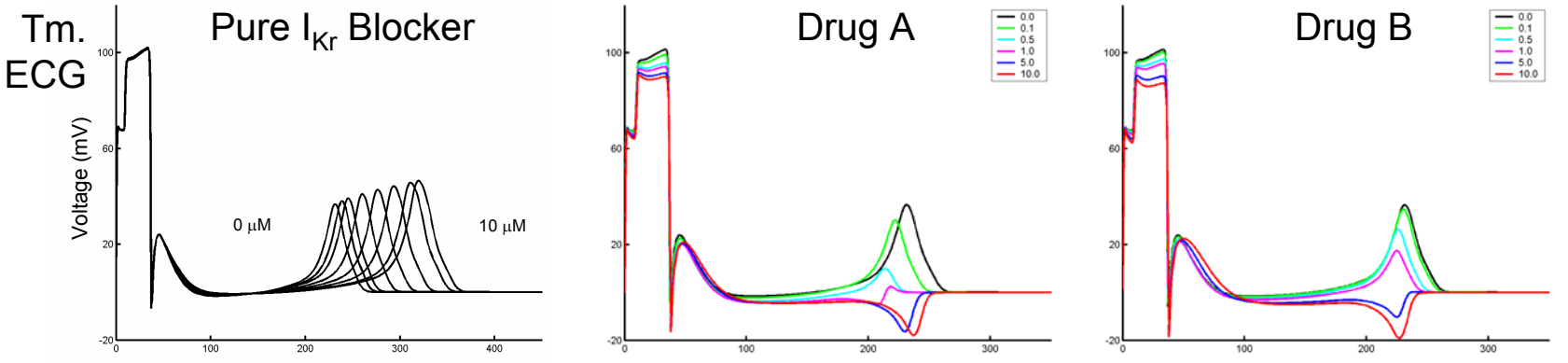
CardioPrism™: Forward-Engineering (II)



- **Pure I_{Kr} blockers prolong the QT interval (left panel)**
- **Both drugs act to shorten the QT interval and reduce the amplitude of the T wave (at high doses there is also inversion)**
- **At higher concentrations of Drug A (5-10 μM), shortening of the QT interval reverses but remains less than control**

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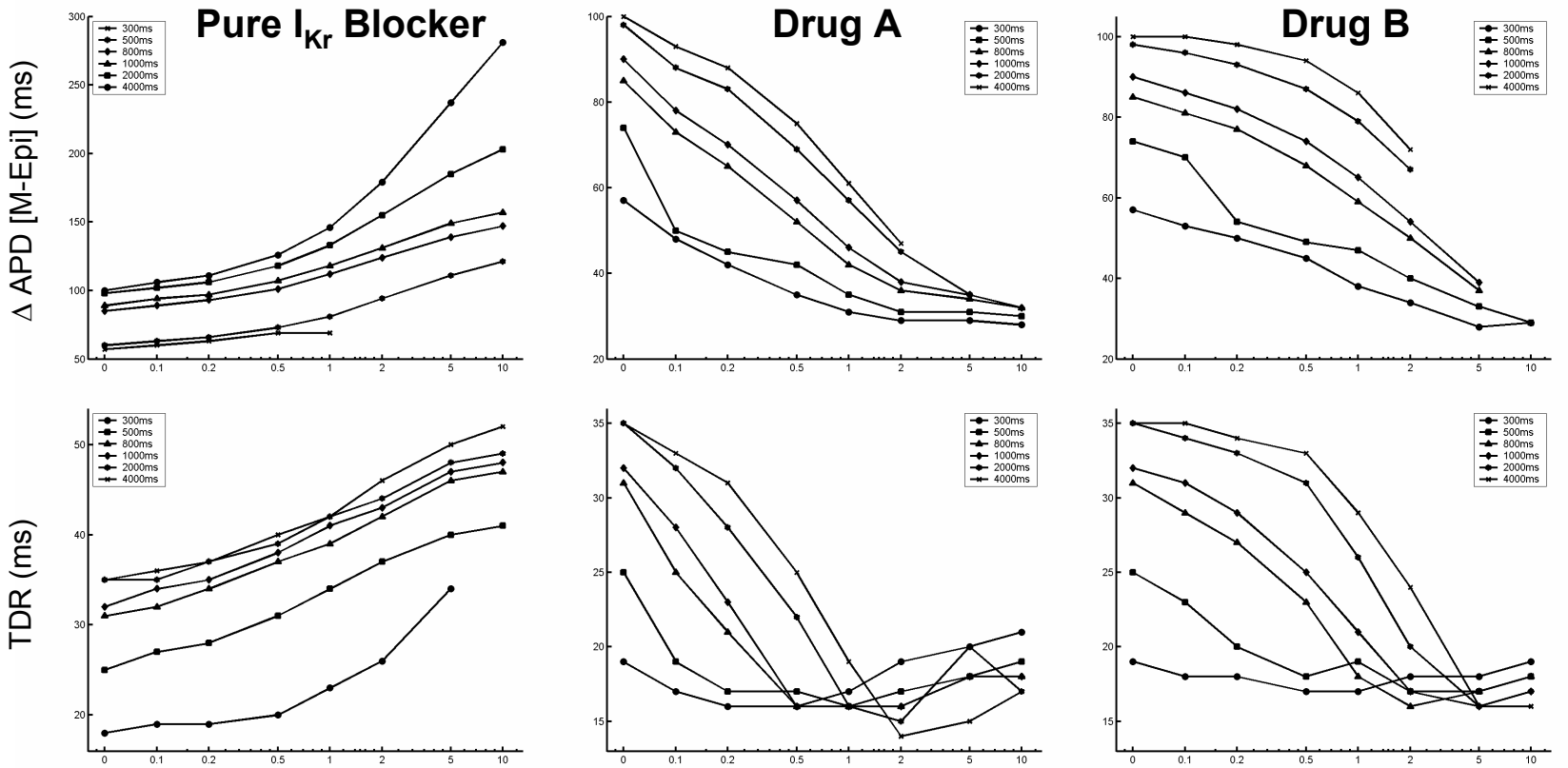
CardioPrism™: Forward-Engineering (III)



- **The difference in APD between isolated epicardial and M cells is, in this example, consistent with the TDR in the 1-D cable**

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CardioPrism™: Conclusions, Drug A vs. Drug B

- **Both compounds block multiple ion currents**
  - Data and model indicate significant block of I_{Kr}, $I_{Ca\text{-}L}$ and $I_{Na\text{-}sus}$
  - Stark contrast to "null-hypothesis" of pure I_{Kr} block
- **No dose-dependent QT prolongation or increase in TDR**
- **Confidence intervals for Drug A smaller *vs.* Drug B**
  - Confidence in predictions is better for Drug A
- **Experimental Confirmation**

Fast I_{Na}	Drug A: IC_{50} = 2.30 µM Drug B: IC_{50} = 4.48 µM
Late I_{Na}	Drug A: IC_{50} = 0.23-0.46 µM Drug B: IC_{50} = 0.45-0.90 µM
Model	Drug A: IC_{50} = 0.27 µM Drug B: IC_{50} = 0.59 µM

Data Set: Drug C
HERG current is strongly inhibited, APD is prolonged

- IC_{50} ($hERG_{HEK293}$) = 28.7 nM
  - IC_{50}'s also available for two metabolites and other compounds in same class

- Detailed AP study (parent + two metabolites)
  - Canine Purkinje Fibers: 2 frequencies, concentration range over 3 orders of magnitude
  - Parent + one metabolite are associated with APD prolongation, at >0.1 µM

CardioPrism™ Conclusions, Drug C

- "*Pure I_{Kr} block*" hypothesis examined again
  - Single cell models
  - Tissue "cable" model
  - NOT satisfactory to explain observed AP's
- Reverse-engineering
  - Both global & local estimation routines could NOT find an IC_{50} profile on the 6 candidate channels (I_{Kr}, I_{Ks}, I_{to}, $I_{Ca\text{-}L}$, $I_{Na\text{-}Ca}$, $I_{Na\text{-}sus}$) to interpret AP data for Drug C in a satisfactory fashion
  - Dose-dependent block of the 6 ion currents do not suffice to predict the action potential data for Drug C
- Need to formulate & test other hypotheses
  - Drug-induced potentiation (rather than inhibition) of channel currents?
  - Additional mechanisms in the Purkinje fiber?

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THANK YOU

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