Using QSP to predict cardiotoxicity caused by cancer drugs

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Outline

Cardiotoxicity caused by tyrosine kinase inhibitor drugs (TKIs)

Integrated experiments & modeling address toxicity mechanisms

- **Assessment of changes in gene expression**
- **Simulations with mechanistic models**
- **Cellular physiology experiments**

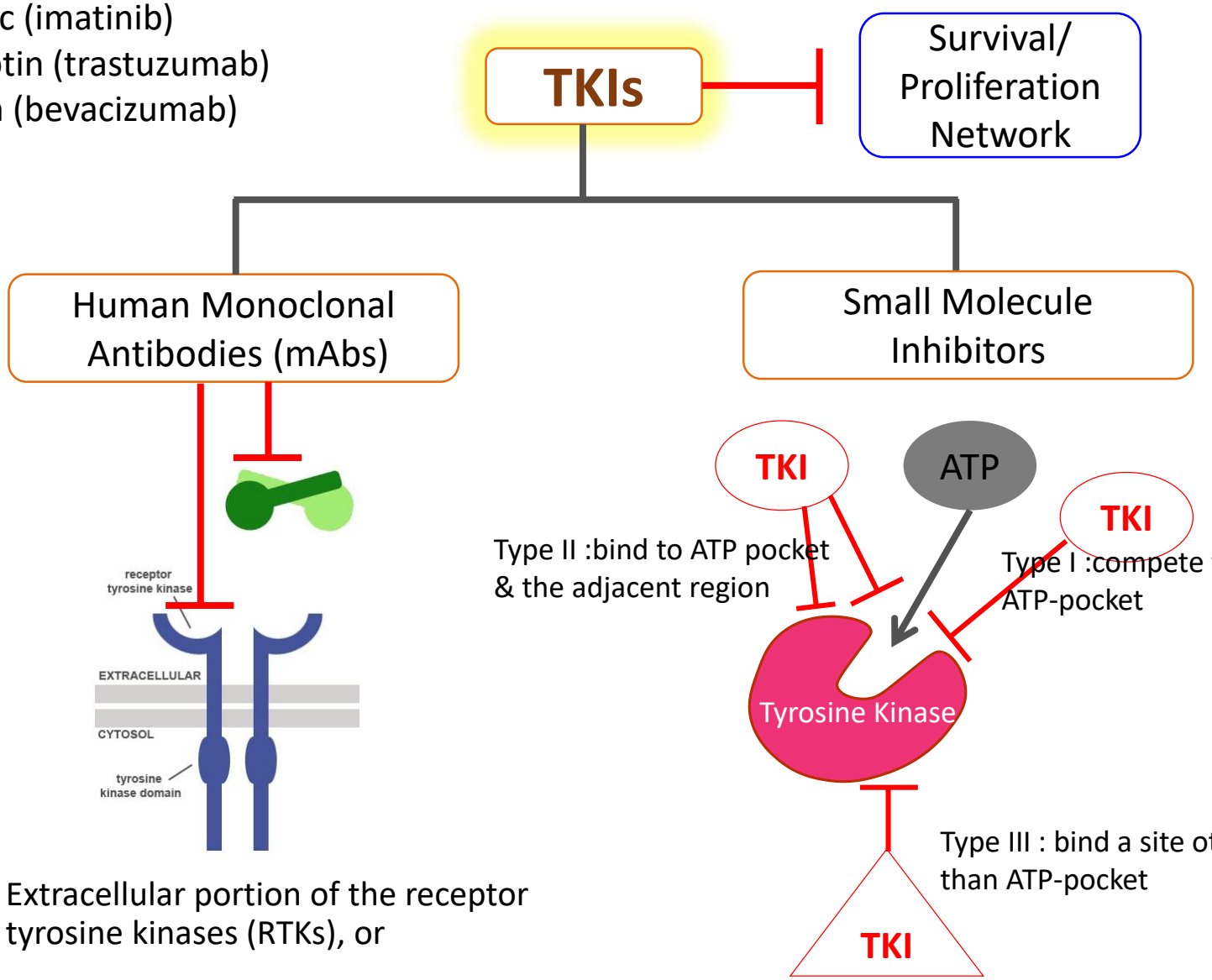
Results: Individual-specific changes in arrhythmia susceptibility caused by drug-induced changes in gene expression

Future directions

Tyrosine Kinase Inhibitors (TKIs)

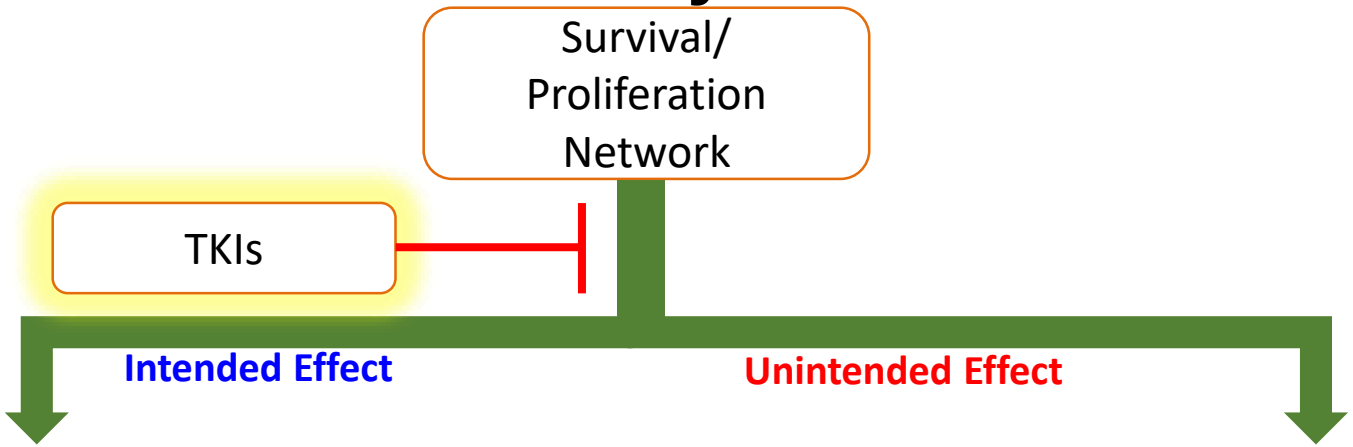
Revolutionary treatments for several cancers

Gleevec (imatinib)
Herceptin (trastuzumab)
Avastin (bevacizumab)

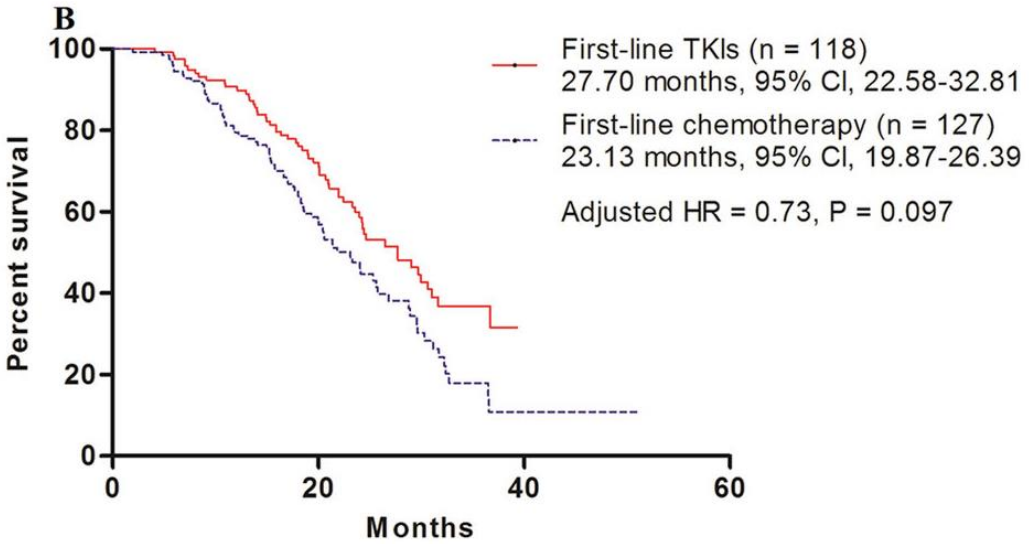


- Extracellular portion of the receptor tyrosine kinases (RTKs), or
- The potential ligands to RTKs

Cardiotoxicity of TKIs

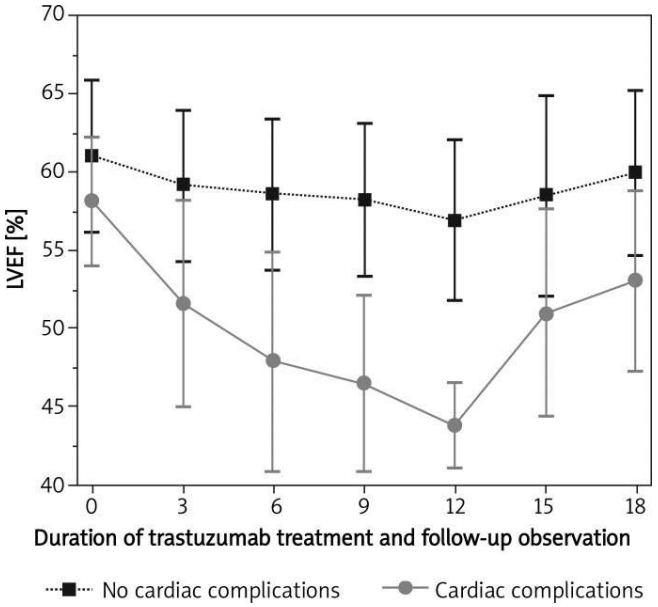


Xu et al. (2016) *Scientific Reports* **6**: 36371 .



- Increased survival rate of cancer patients
- Proven to be highly effective cancer treatment

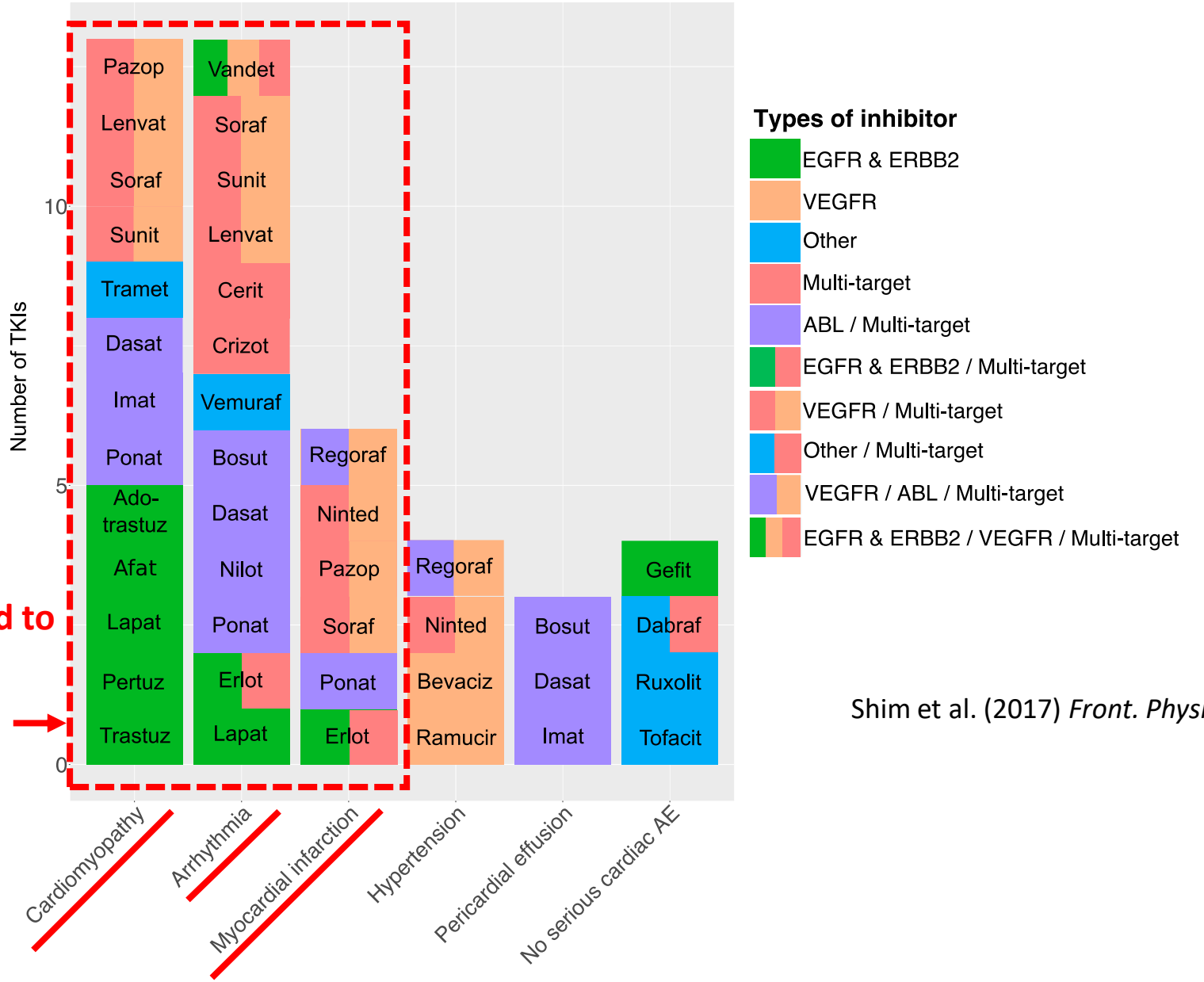
Piotrowski et al. (2012) *AMS* 8:227-235.



- Serious cardiac side effect

Mechanisms underlying cardiotoxicity are poorly understood

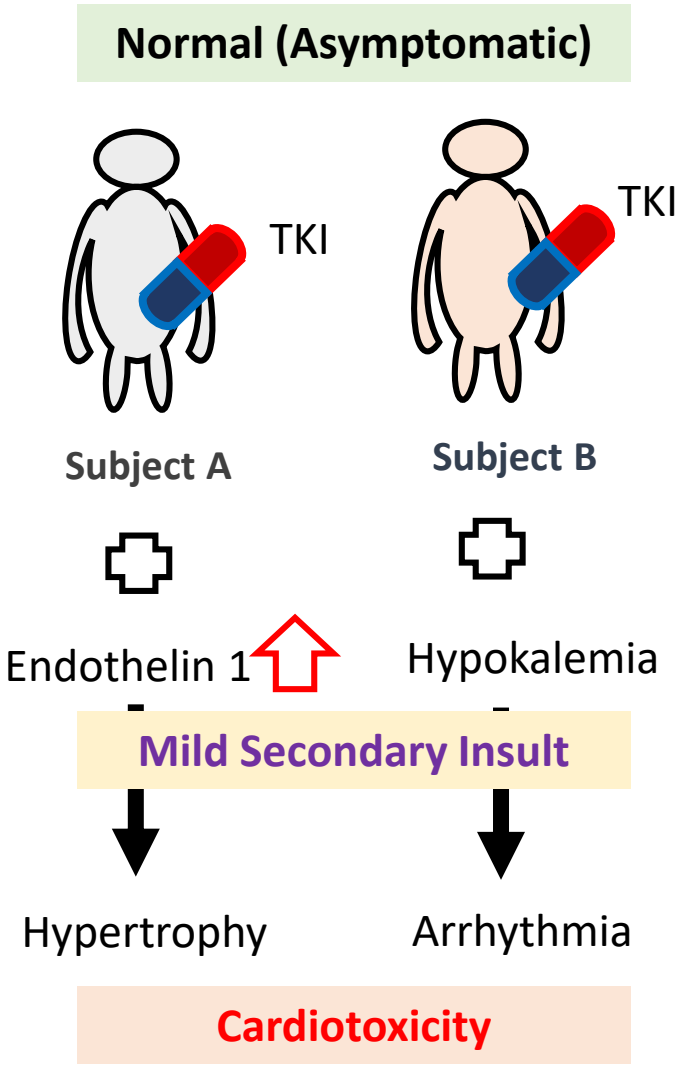
Many TKIs cause cardiotoxicity



Pathology related to direct toxicity on cardiomyocytes

Shim et al. (2017) *Front. Physiol.* 8:651.

Goal: elucidate patient-specific cardiotoxicity mechanisms

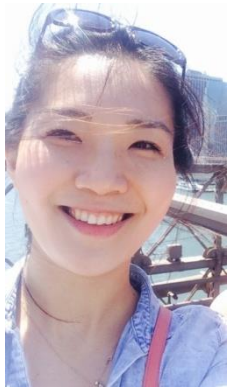


Assumption

- Applying high drug concentrations to kill myocytes is a poor toxicity model

Hypothesis

- "Two-hit." TKIs may alter gene expression in myocytes such that cells become susceptible to additional insults
- Drug responses may be specific to cell lines from particular individuals



Jaehee Shim
PhD 2019
Now at Applied Biomath

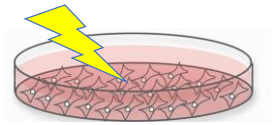
See also: Shim et al. (2017) *Front Physiol* 8:651.

Approach

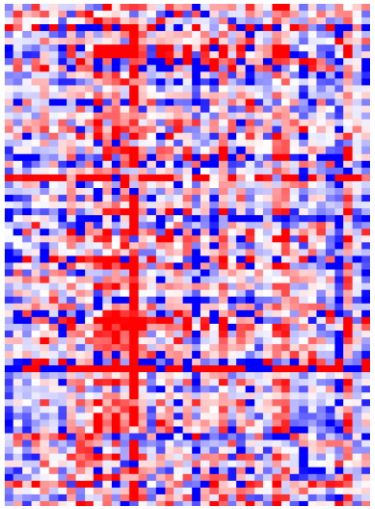
Step 1: integrate gene expression data with mechanistic mathematical models to generate predictions

Step 2: test predictions experimentally to support or refute hypotheses

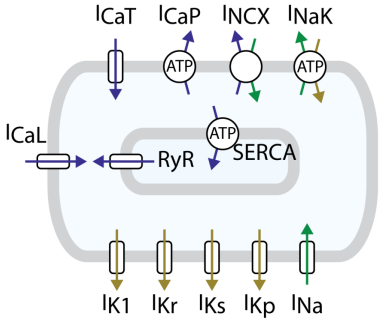
Screening in cell culture
TKI-treated myocytes
(iPSC-CMs)



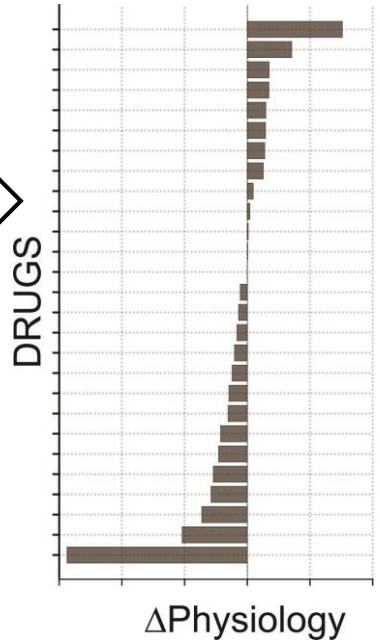
Gene expression measurements
mRNA-seq
26 drugs, 2 cell lines



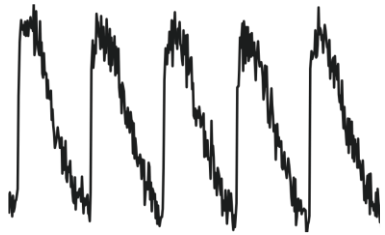
Mechanistic mathematical modeling
Systems of ODEs



Predictions



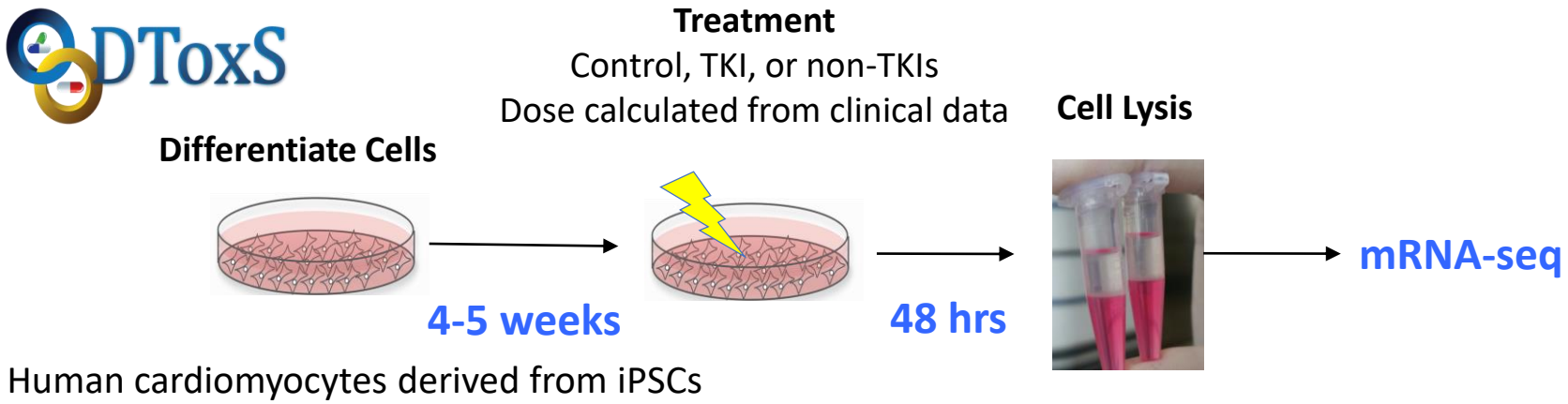
Experimental tests



Methodological Details

- How do we obtain the gene expression data?
- What mathematical models do we use?
- What are the experimental tests?

Experimental Design for Gene Expression Data



Drug Treatments

Protein kinase inhibitors – many with cardiac risk

AFATINIB	NILOTINIB	DASATINIB	TOFACITINIB
AXITINIB	PAZOPANIB	ERLOTINIB	TRAMETINIB
BOSUTINIB	PONATINIB	GEFITINIB	VANDETANIB
CABOZANTINIB	REGORAFENIB	IMATINIB	VEMURAFENIB
CERITINIB	RUXOLITINIB	LAPATINIB	CETUXIMAB
CRIZOTINIB	SORAFENIB	TRASTUZUMAB	BEVACIZUMAB
DABRAFENIB	SUNITINIB	RITUXIMAB	

Mount Sinai DToxS Center

NIH LINCS PROGRAM

LINCS = Library of Integrated Network-based Cellular Signatures

The Mount Sinai LINCS Team

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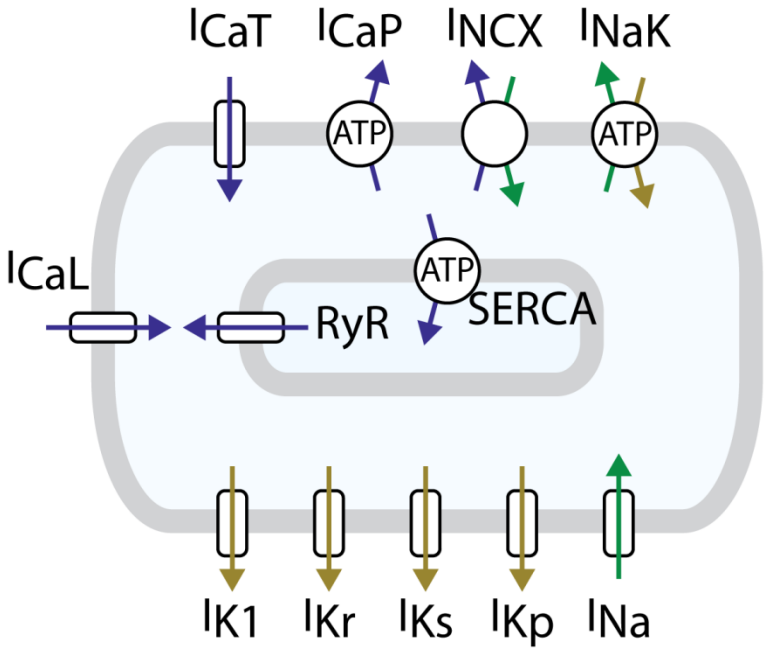
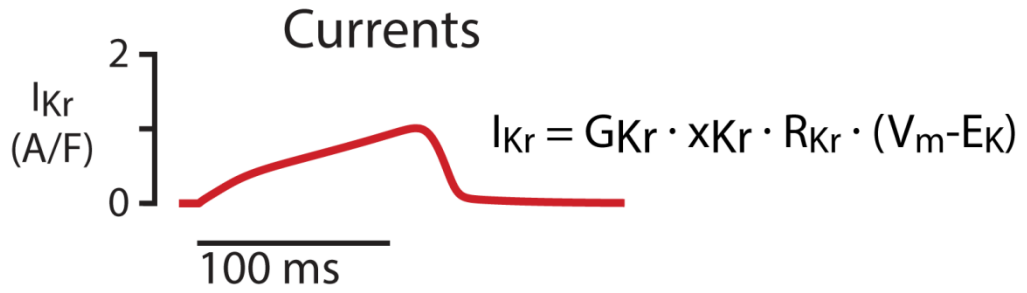
Mechanistic cardiac myocyte models

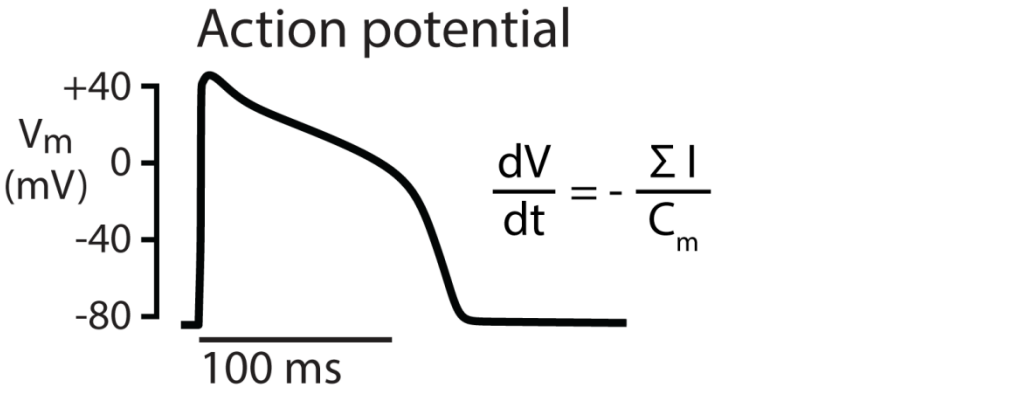
Models simulate ionic currents, intracellular ionic homeostasis

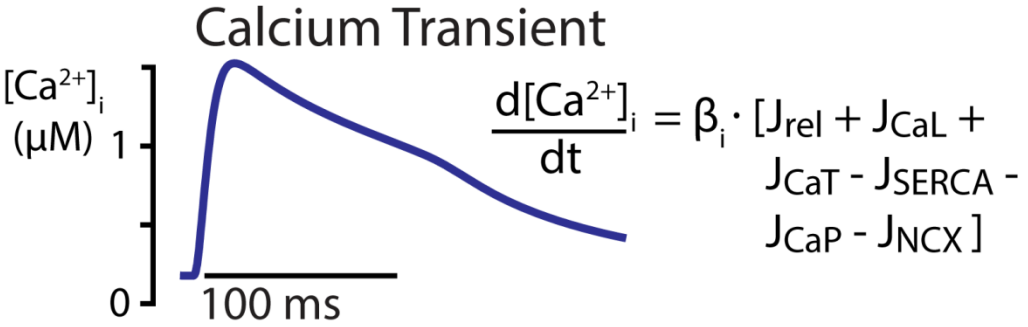
Models have been developed over ~50 years of basic physiology research



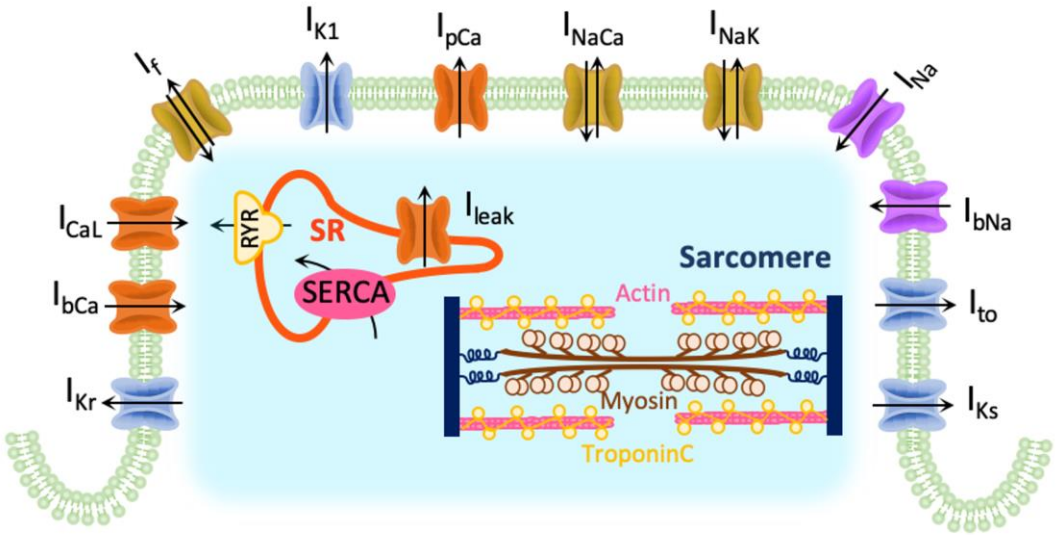
- 10-20 ion channels, pumps, and transporters
- 20-60 ordinary differential equations
- Drug effects simulated by reducing/enhancing activities







Pipeline: patient-specific predictions based on transcriptomic data



Electrophysiology: Paci et al *Ann BME* 2013
Contraction: Rice et al *Biophys J* 2008

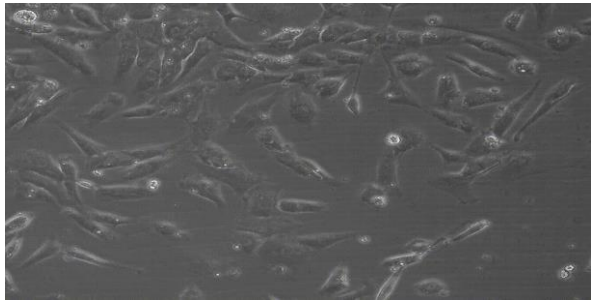
Assumptions:

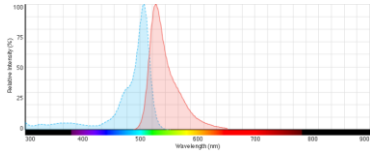
- Model parameters correspond to defined genes
- mRNA levels are proportional to activities

Parameter	Genes
G_{Na}	SCN5A
G_{CaL}	CACNA1C * all voltage gated calcium channel * CACNA1S,CACNA1D,CACNA1B,CACNA1I,CACNA1G,CACNA1H, CACNA1A, CACNA1E,CACNA1F,CACNA1C,CACNA2D1
G_{to}	KCND2, KCND3, KCNA4, KCNA7
G_{Ks}	KCNQ1, KCNE1
G_{Kr}	KCNH2
G_{K1}	KCNJ2, KCNJ12
P_{NaK}	ATP1A1
I_{up}	ATP2A2
G_{pCa}	ATP2B4
G_f	HCN2, HCN4
K_{NaCA}	SLC8A1
Troponin	TNNC1
Myosin	MYH6, MYH7
Actin	ACTC1

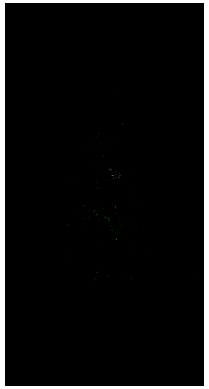
Methods for experimental tests

1- Stem cell derived cardiomyocytes (iPSC-CMs)

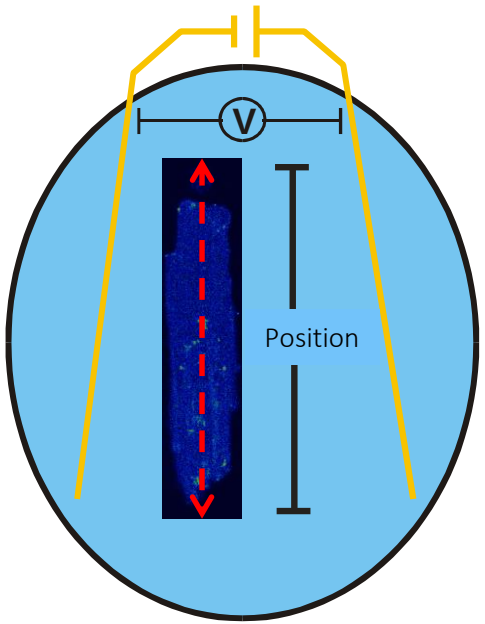




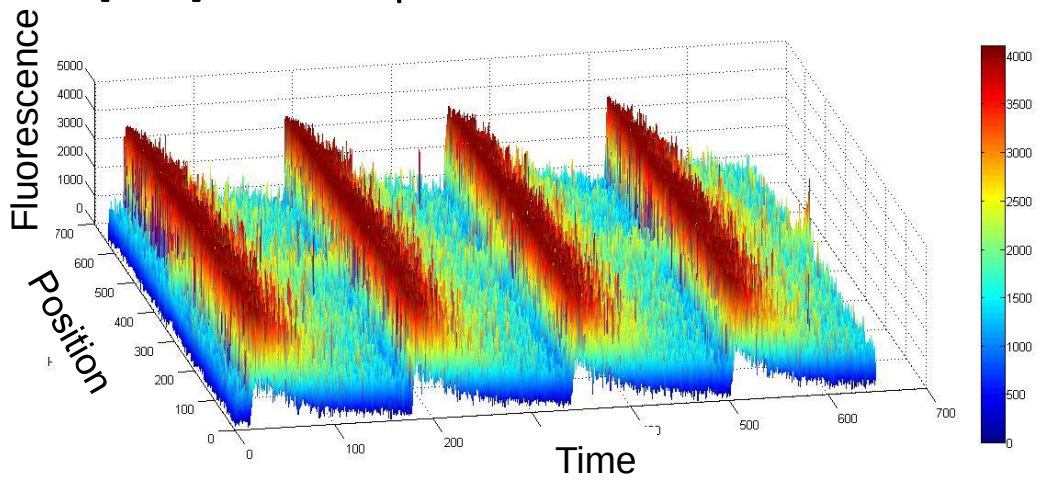
Fluo3-AM
FluoVolt



2- Electrically stimulate cells



3- Record [Ca^{2+}] or action potentials as function of location and time

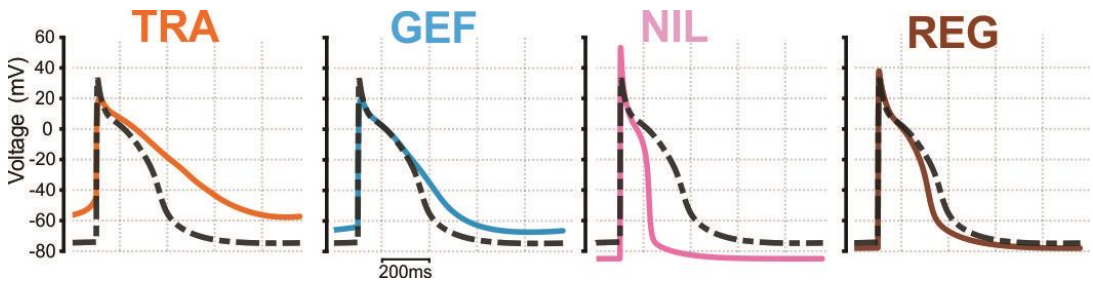


Why integrate Omics data with mechanistic models?

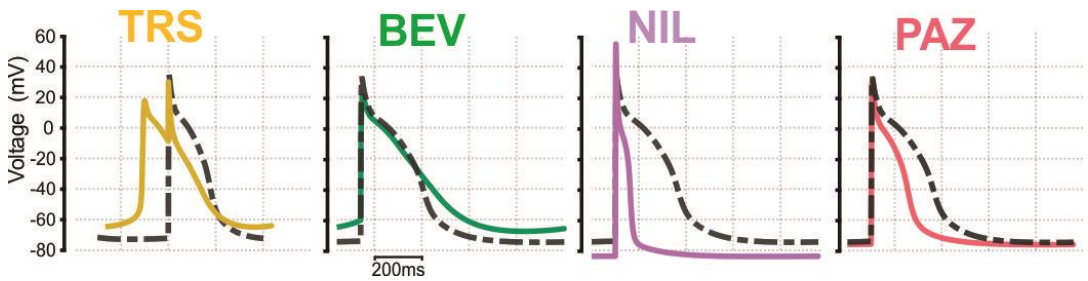
- Omics measurements are generally snapshots. Simulations can predict dynamics.
- Simulations both generate predictions and suggest prioritization of experiments.

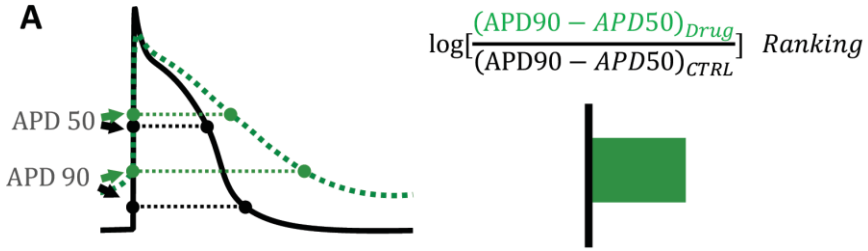
Individual-specific predictions of altered electrophysiology

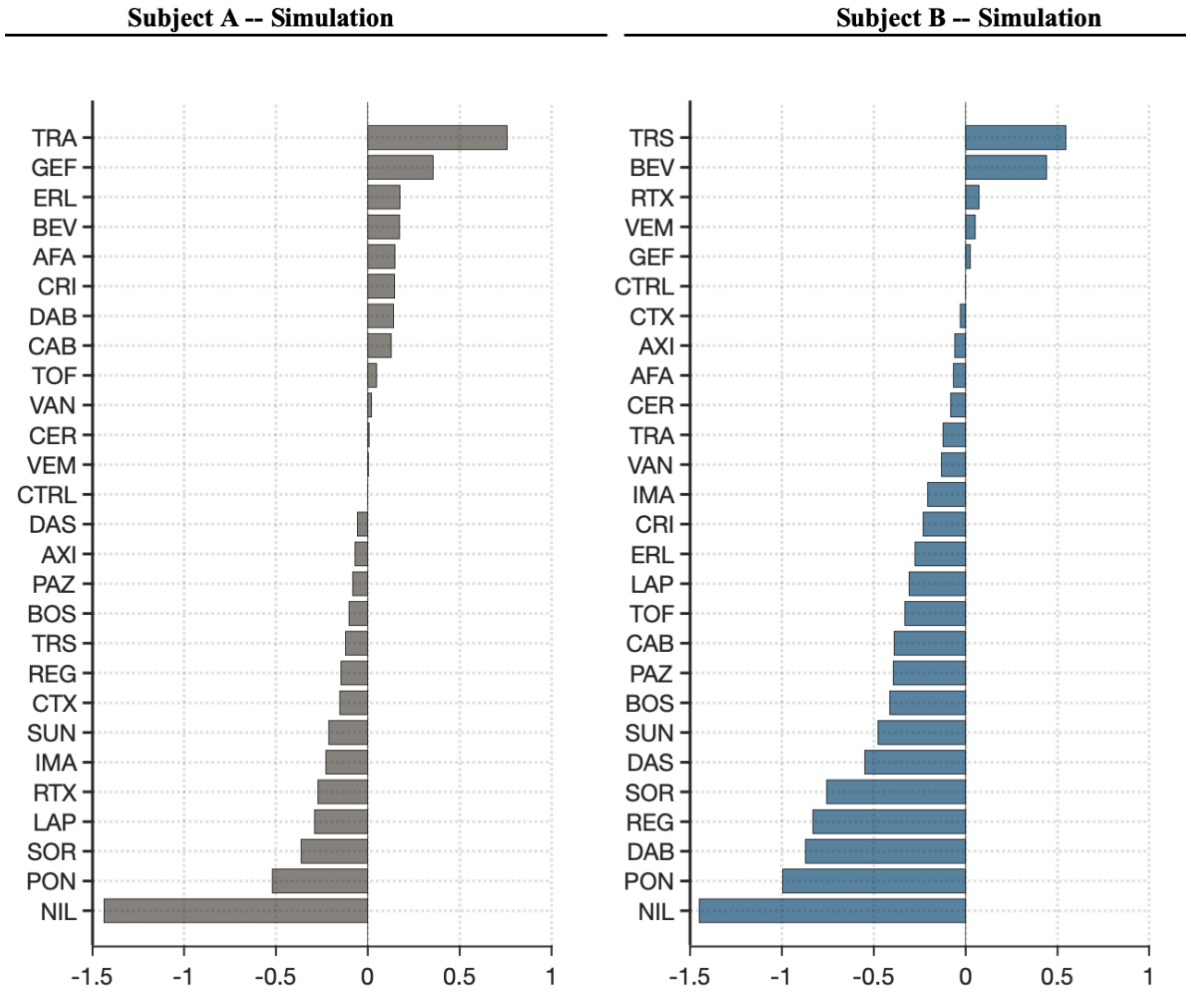
Cell Line A



Cell Line B





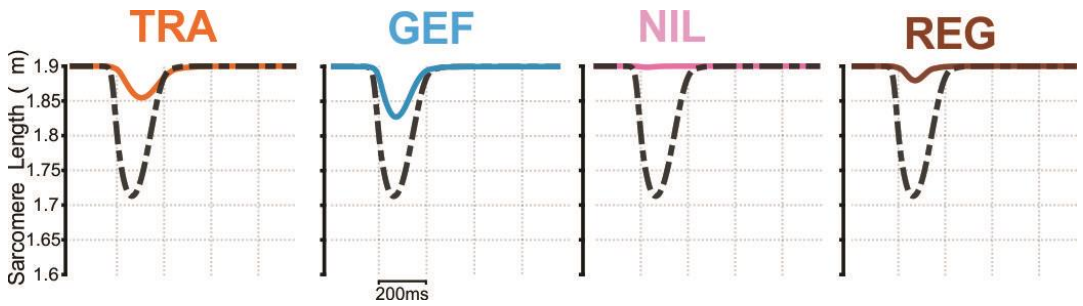


Action potential triangulation

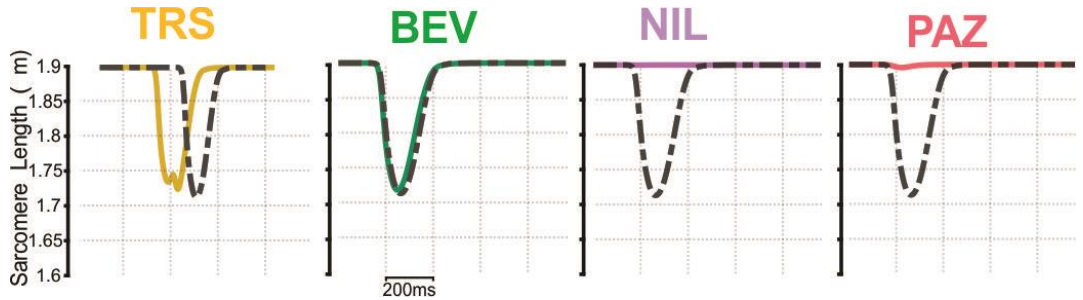
Spearman rank correlation ρ=-0.16

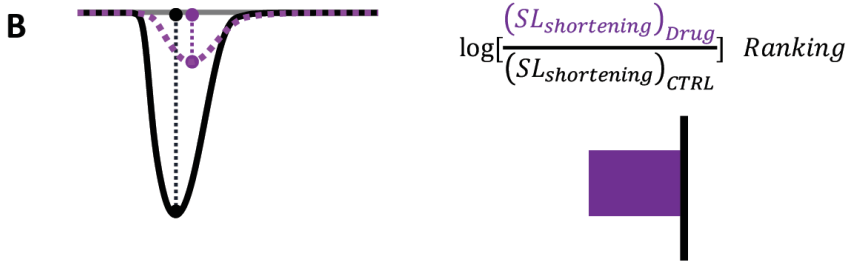
Individual-specific predictions of altered contraction

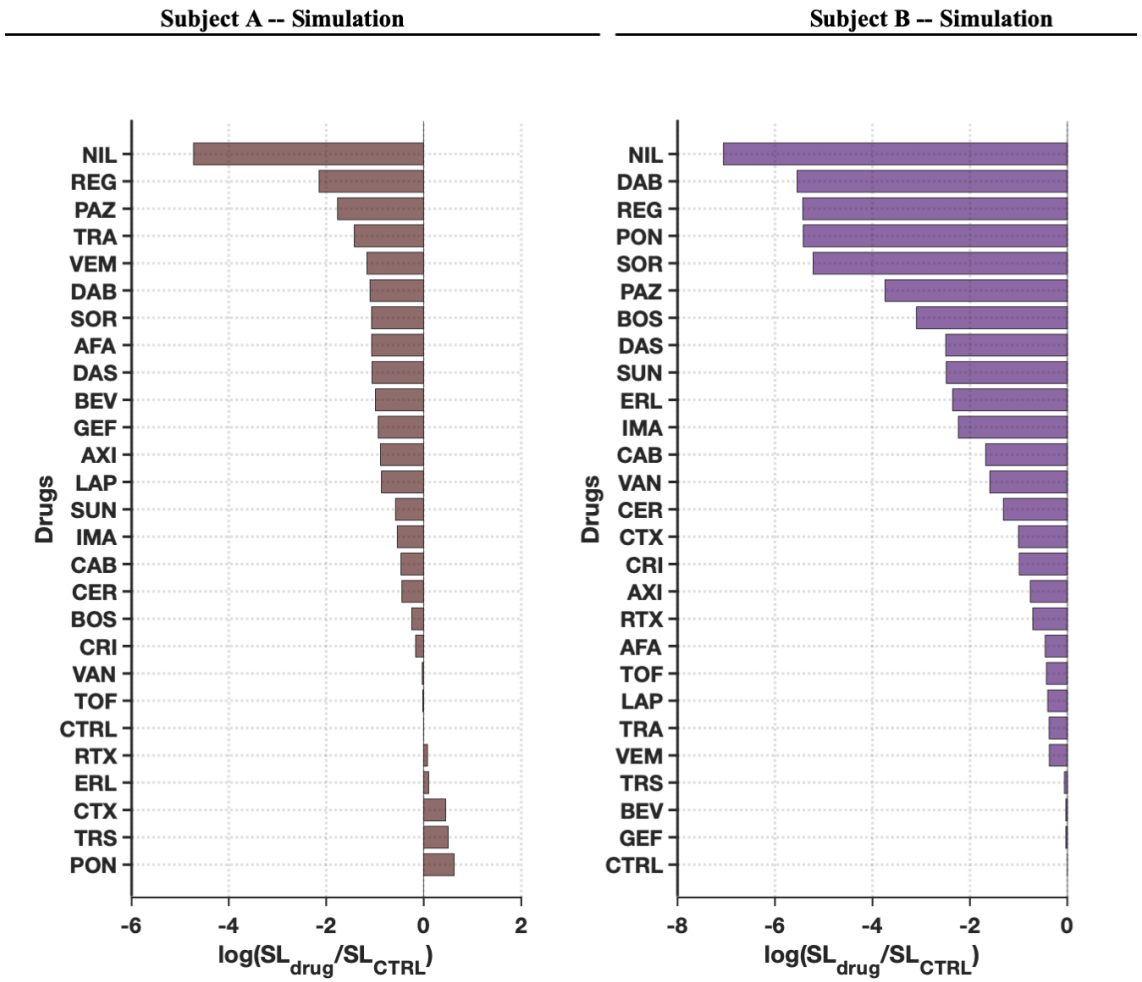
Cell Line A



Cell Line B







iPSC-CM contraction

Spearman rank correlation ρ=0.64

Which modeling predictions should we test?

Simulations allow for efficient prioritization

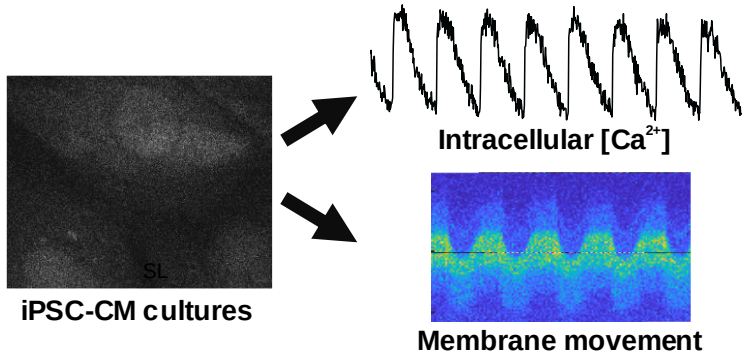
(1) Drugs that are predicted to have meaningful effects

(2) Drugs that influence both electrophysiology and contraction

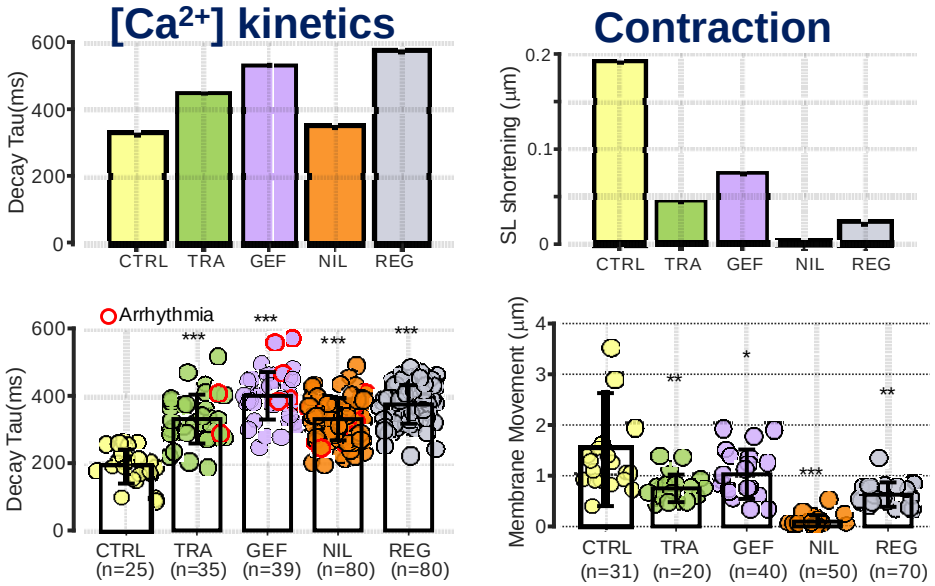
(3) Drugs whose effects are predicted to differ between cell lines

Drugs were selected based on these criteria

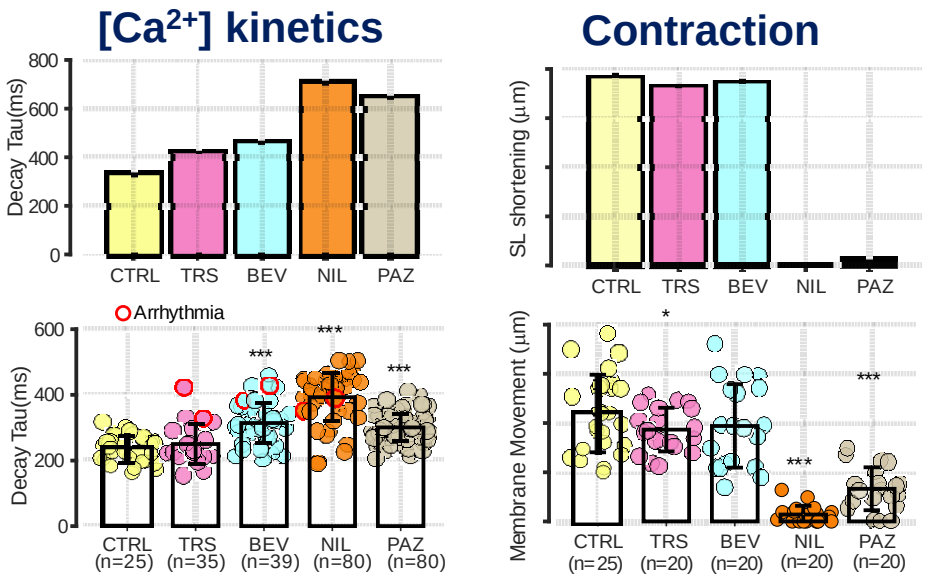
Experimental tests of individual-specific predictions

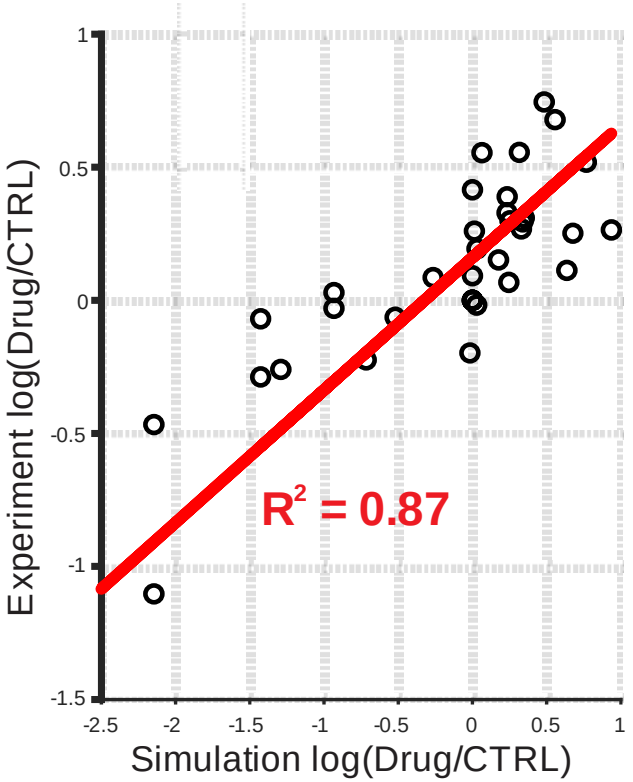


Cell Line A



Cell Line B





4 metrics:

- [Ca^{2+}] decay time constant
- Contraction
- Ca^{2+} transient triangulation
- [Ca^{2+}] area under the curve

4 metrics x 4 drugs x 2 cell lines

Why integrate Omics data with mechanistic models?

- Omics measurements are generally snapshots. Simulations can predict dynamics.
- Simulations both generate predictions and suggest prioritization of experiments.
- Simulations can predict effects of drugs in combination, or of a TKI plus a physiological stimulus (β-adrenergic stimulation, angiotensin, stretch, etc.).

Simulation and experimental protocol

Step 1: Implement Drug-induced changes in gene expression

Simulate drug-induced alterations to action potentials and [Ca^{2+}]

Step 2: Apply pathological stimuli

Predict changes in cellular susceptibility to arrhythmia triggers

Rank drugs for testing based on simulation results

Step 3: Test selected modeling predictions

Measure arrhythmia susceptibility in myocytes derived from stem cells

Important note: Both predictions and experimental tests are cell line-specific

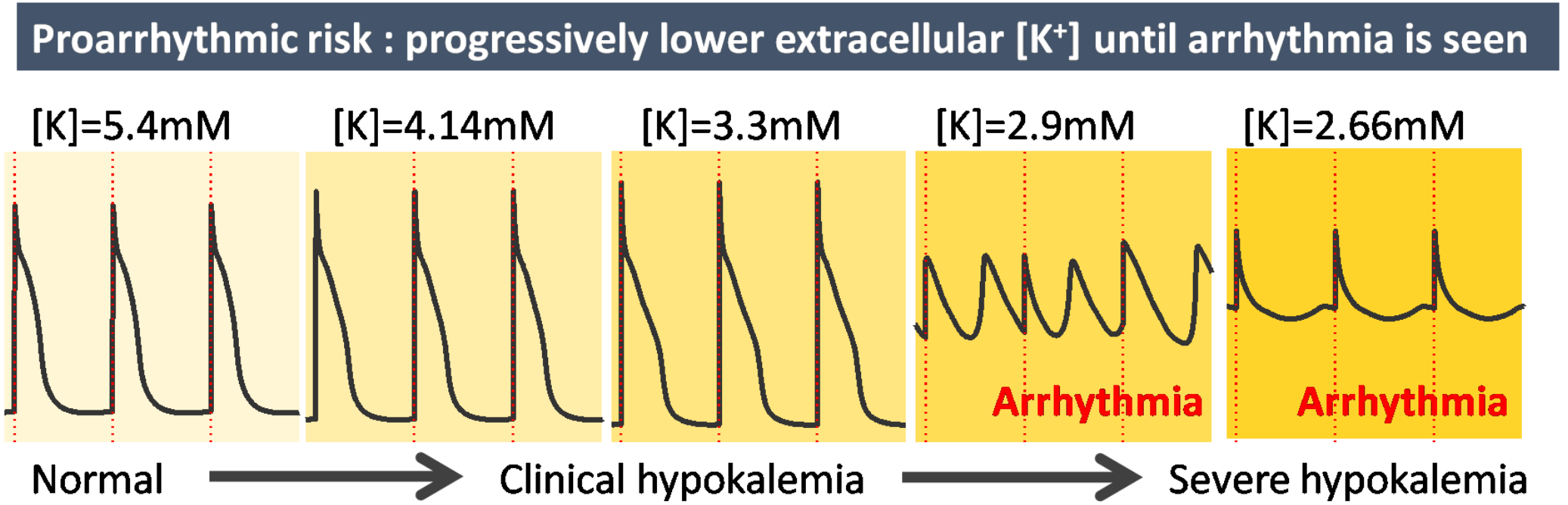
Overall theme: 48 hours of drug treatment does not induce overt toxicity, but can influence susceptibility to additional signals

Step 2: Pathological triggers

Most TKIs are not considered cardiac ion channel blockers

Hypothesis: Gene expression changes may alter susceptibility to arrhythmia triggers

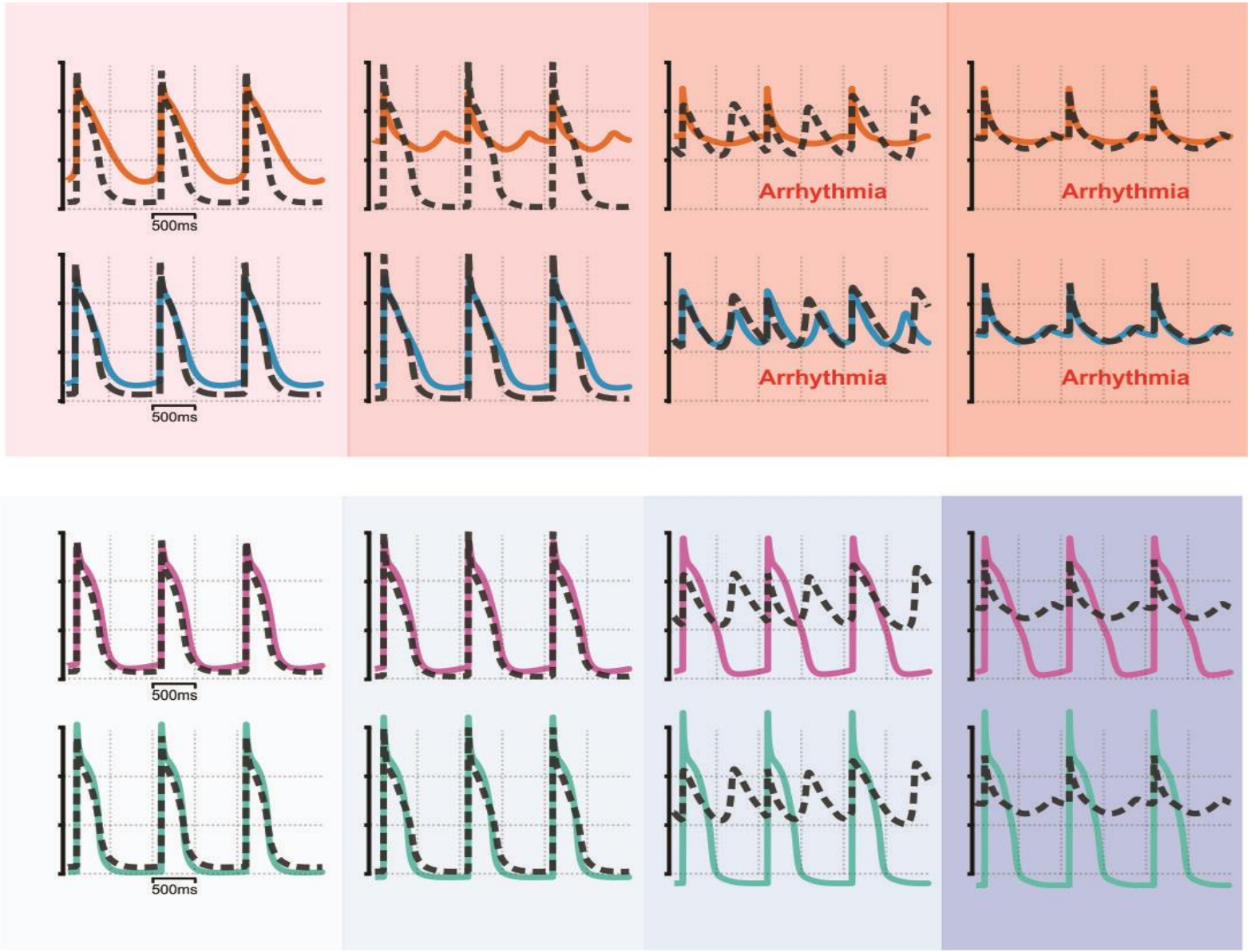
Protocol: simulated hypokalemia



Effects of all TKIs were simulated; interesting predictions were selected for testing

Predictions are cell-line dependent: Cell line 1

$[K^+]$ = 5.4 mM $[K^+]$ = 4.1 mM $[K^+]$ = 2.9 mM $[K^+]$ = 2.5 mM



Trametinib

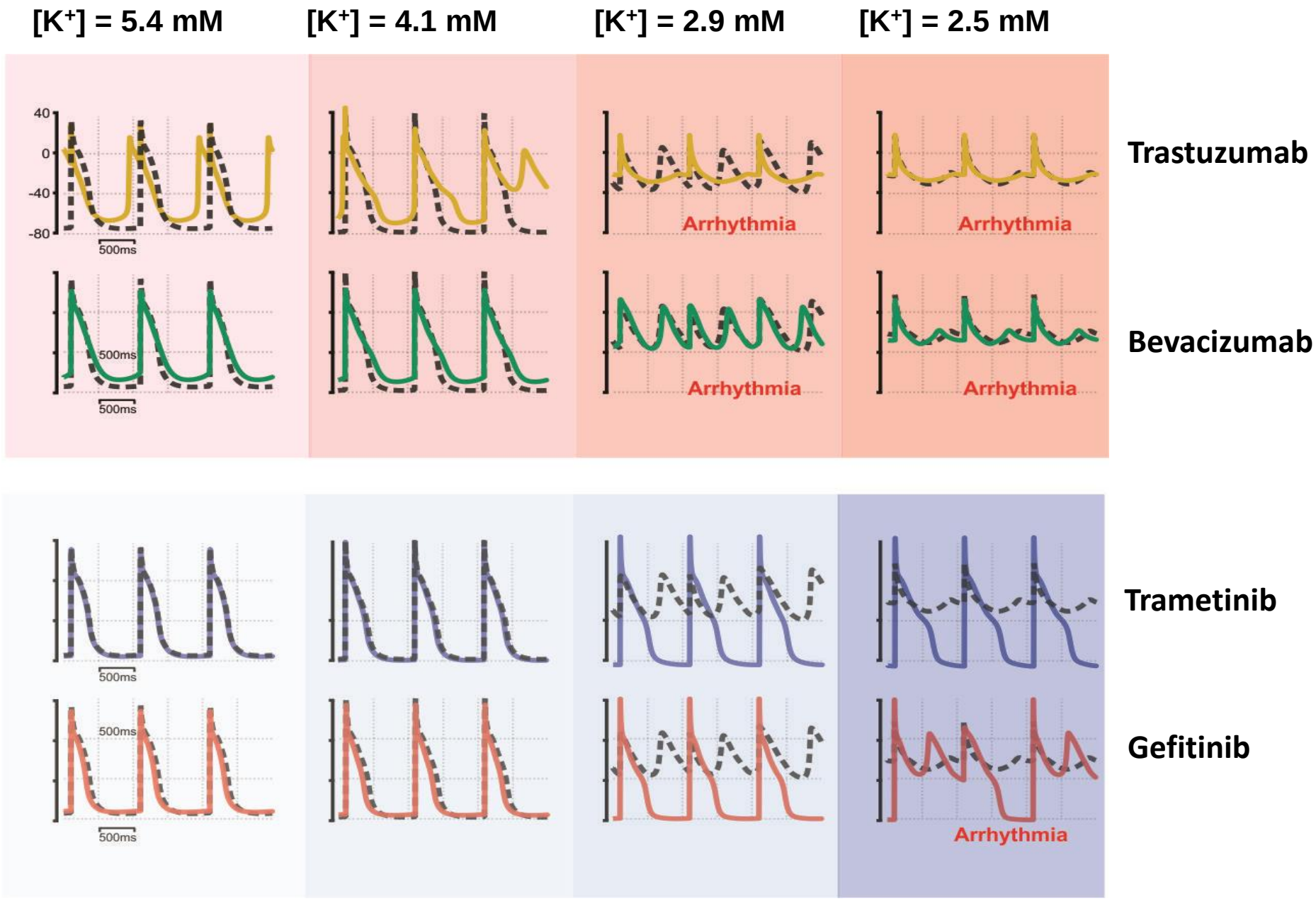
Gefitinib

Trastuzumab

Ponatinib

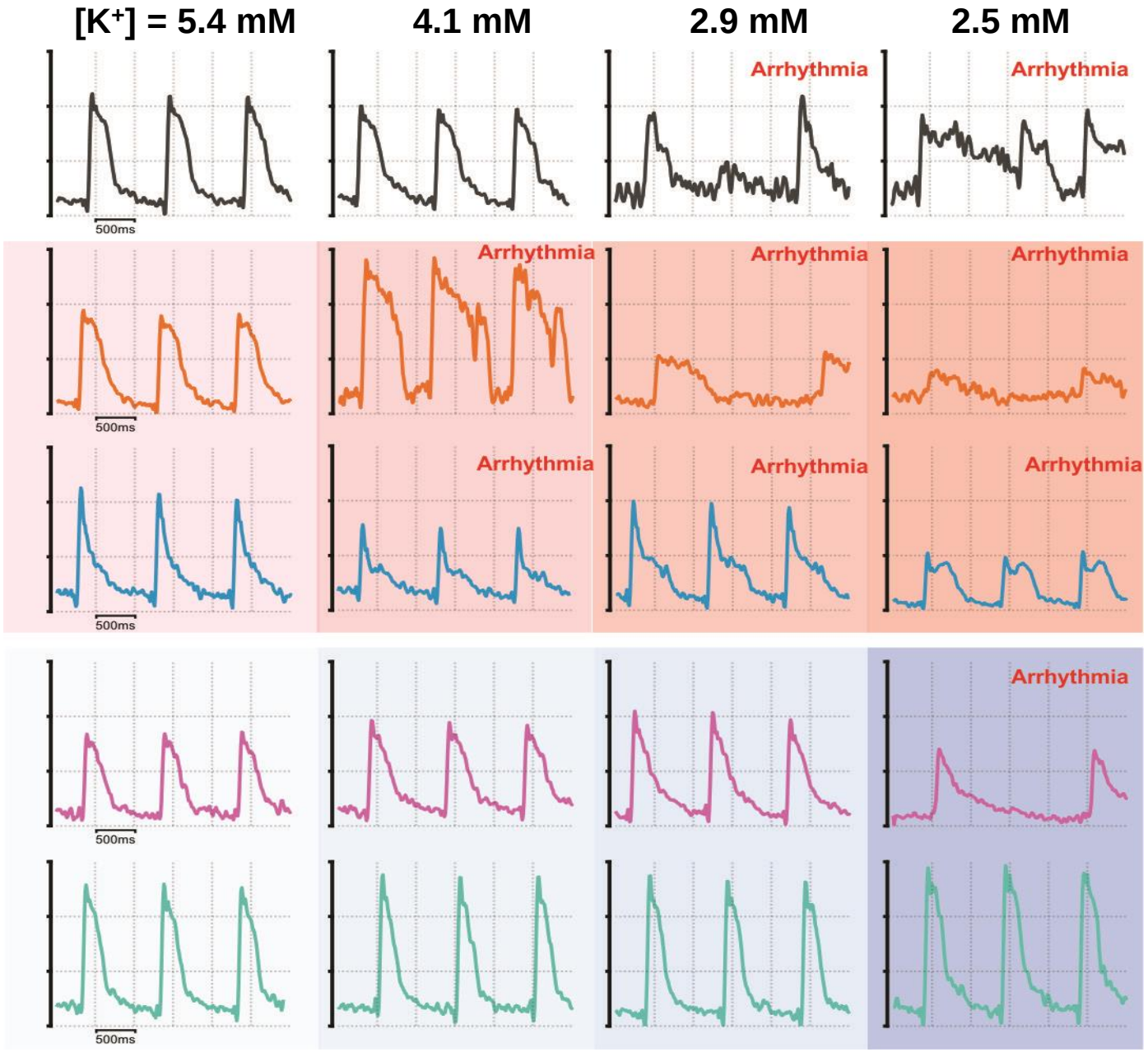
Subject A: Trametinib & Cabozatinib are *toxic;* Trastuzumab & Ponatinib are *protective*

Predictions are cell-line dependent: Cell line 2



Subject B: Trastuzumab & Bevacizumab are *toxic;* Trametinib & Gefitinib are *protective*

Experimental tests: Cell line 1



DMSO Control

Trametinib

↑ susceptibility, consistent with model predictions

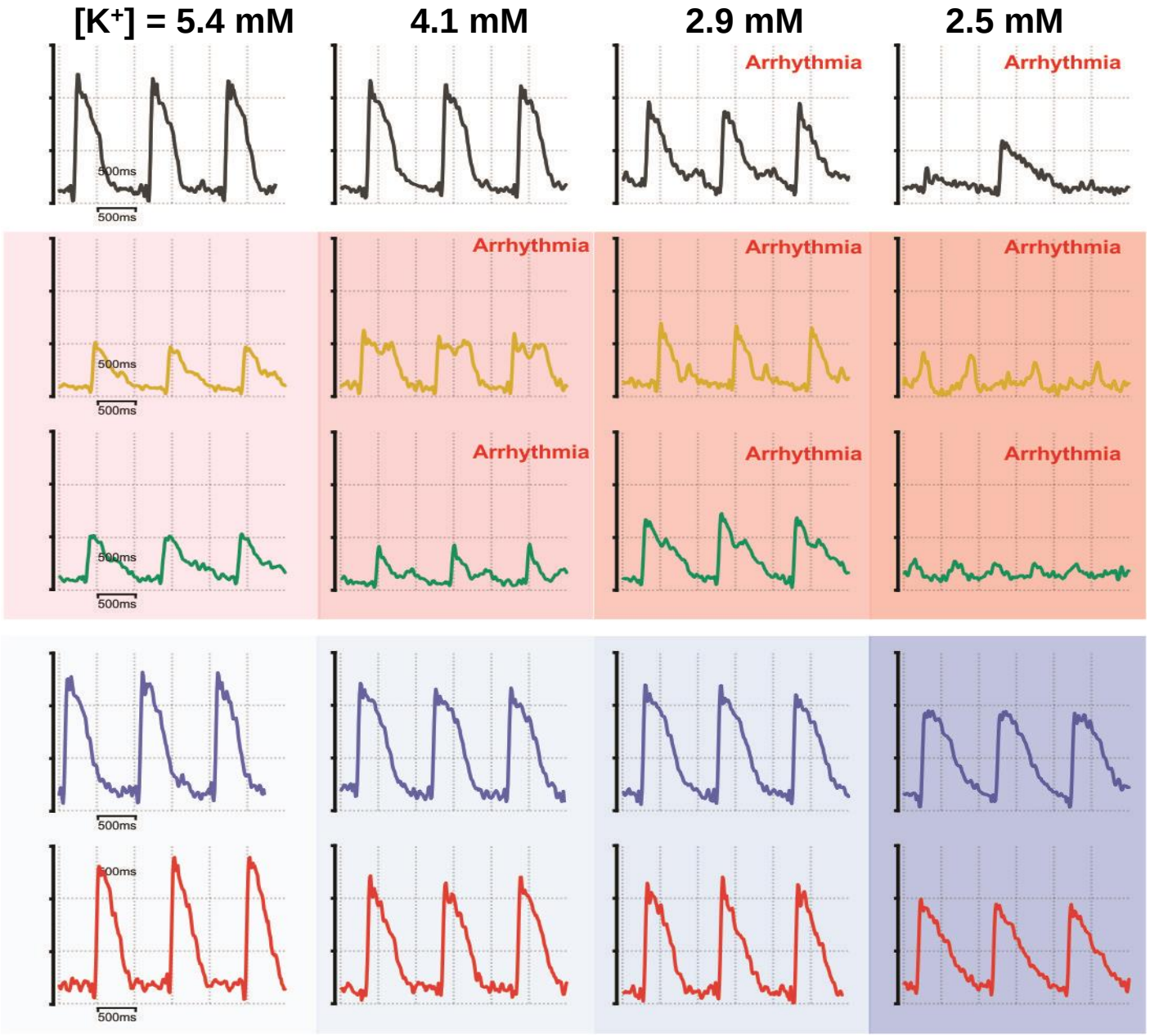
Cabozatinib

Trastuzumab

↓ susceptibility, consistent with model predictions

Ponatinib

Experimental tests: Cell line 2

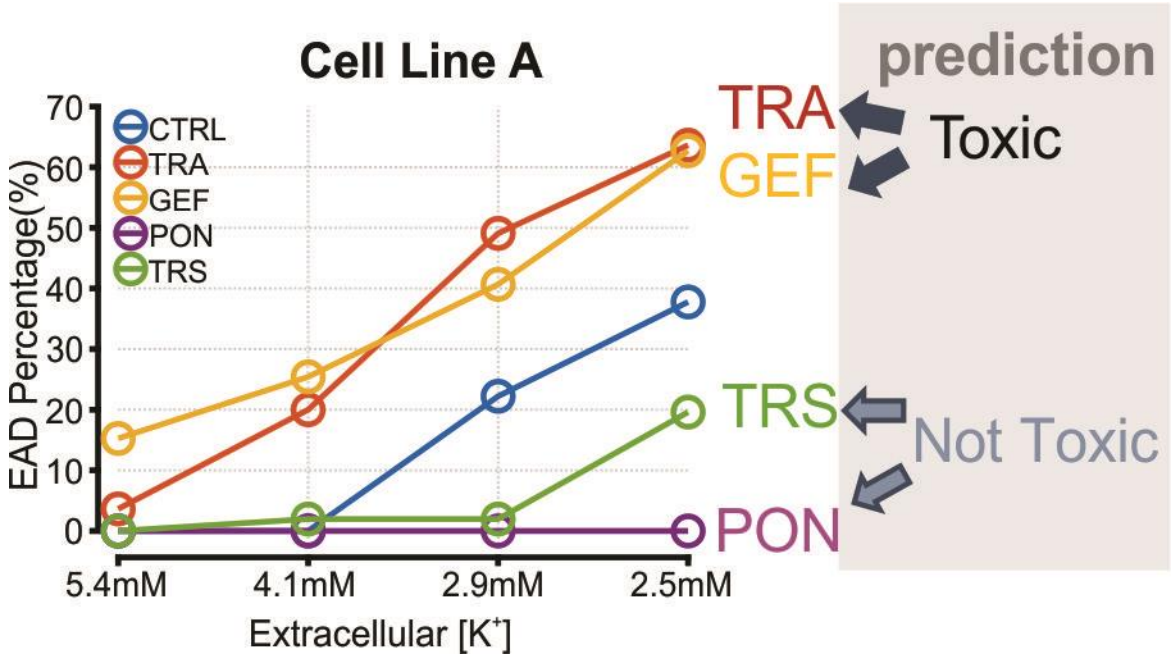


↑ susceptibility, consistent with model predictions

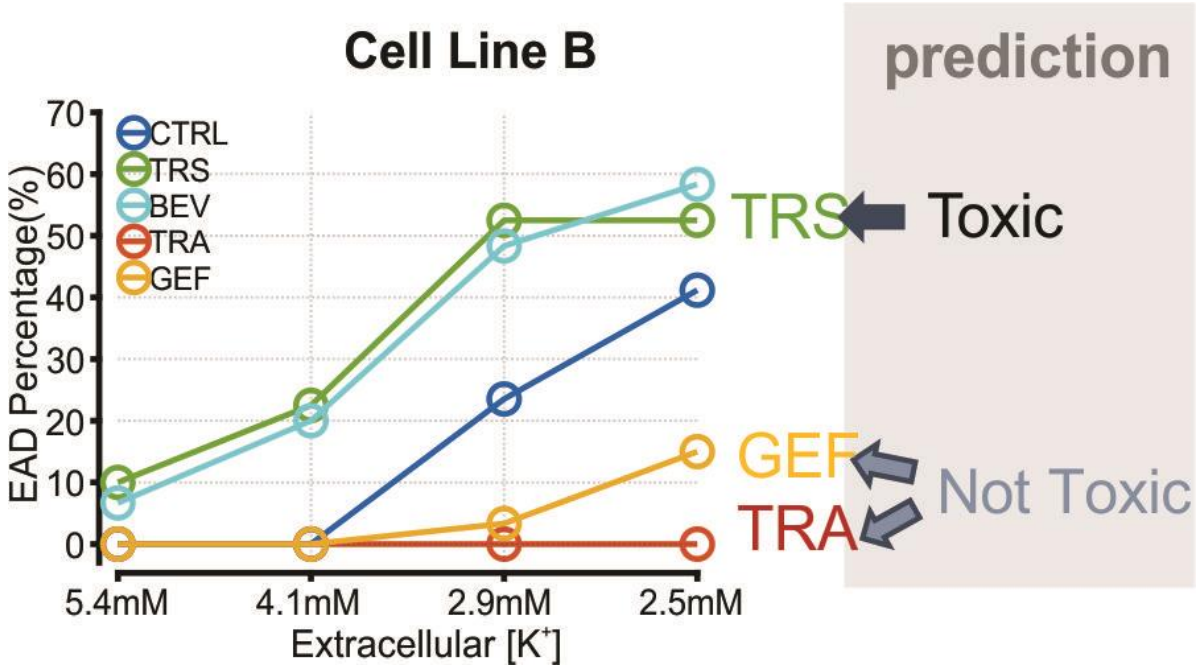
↓ susceptibility, consistent with model predictions

Hypokalemia summary data: Arrhythmia susceptibility

Subject A: Trametinib & Gefitinib are *toxic*
Trastuzumab & Ponatinib are *protective*



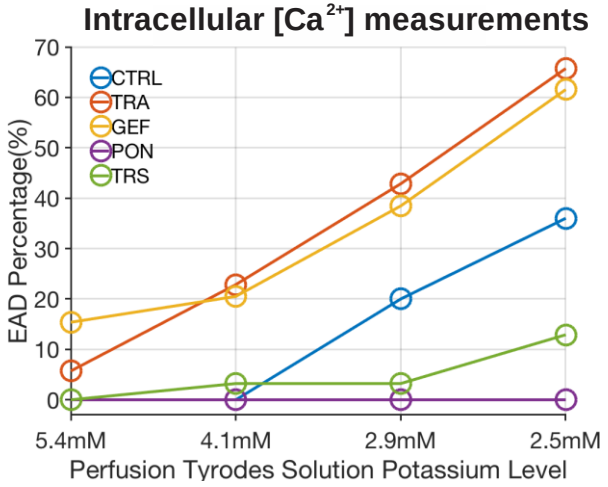
Subject B: Trastuzumab & Bevacizumab are *toxic*
Trametinib & Gefitinib are *protective*

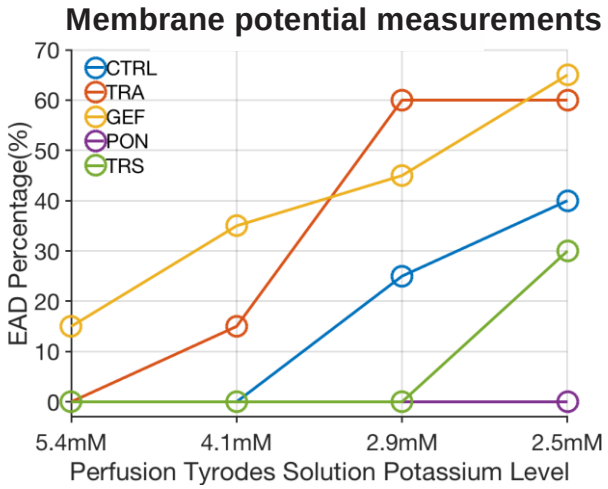


Hypokalemia data: Reproducibility of experiments

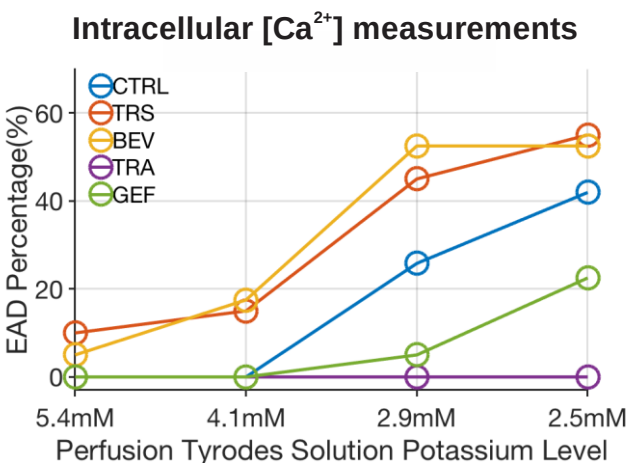
iPSC-CMs can be idiosyncratic. Did we get lucky with particular cell differentiations?

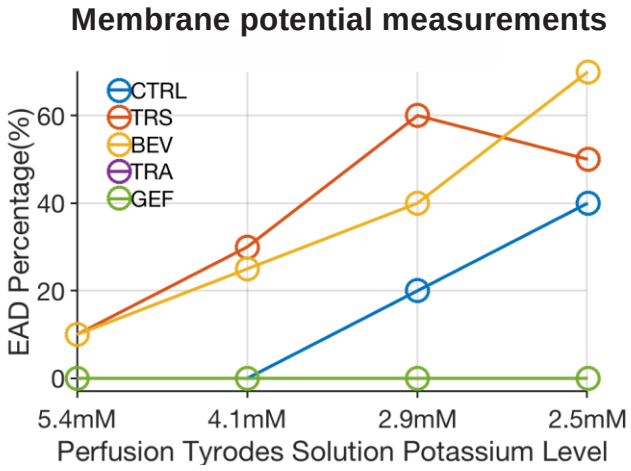
Cell Line A





Cell Line B





[Ca2+] and action potential experiments were performed 3-6 months apart

Why integrate Omics data with mechanistic models?

- Omics measurements are generally snapshots. Simulations can predict dynamics.
- Simulations both generate predictions and suggest prioritization of experiments.
- Simulations can predict effects of drugs in combination, or of a TKI plus a physiological stimulus (β-adrenergic stimulation, angiotensin, stretch, etc.).
- Modeling results can suggest mechanisms underlying differences between drugs or drug classes.

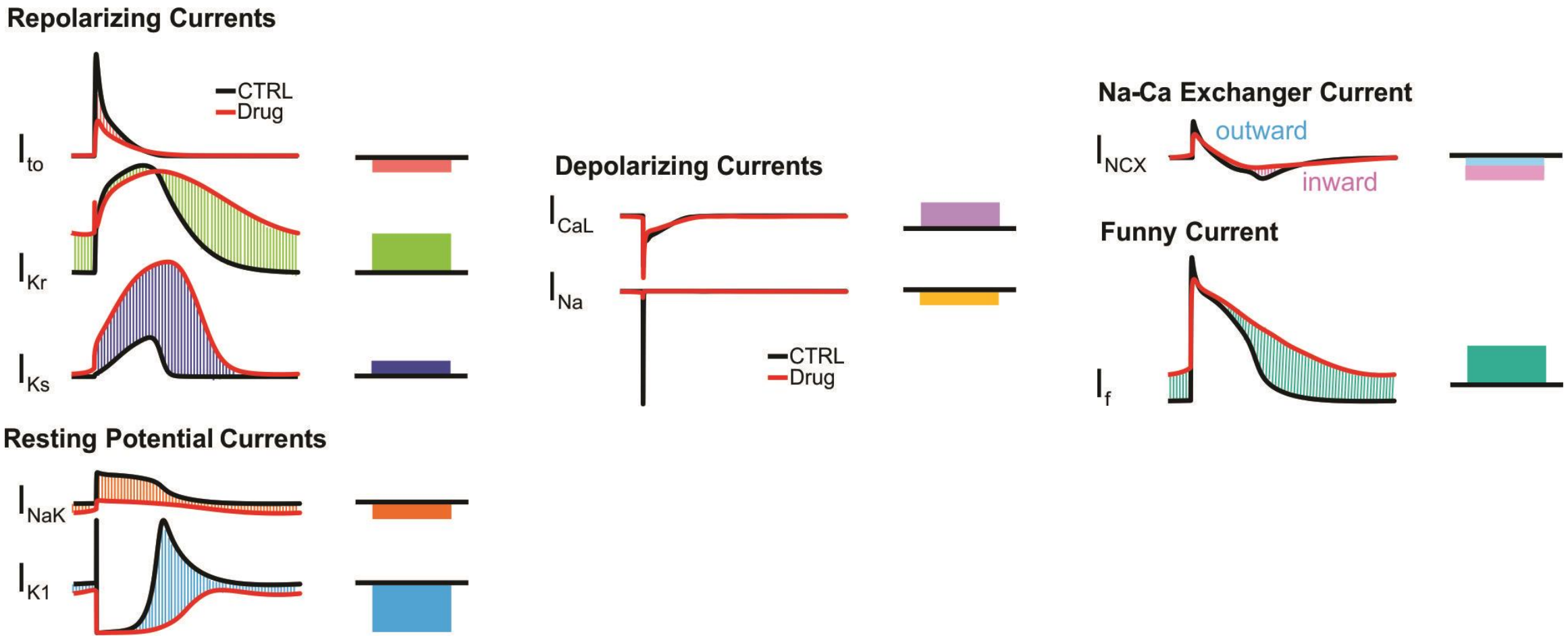
Why integrate Omics data with mechanistic models?

- Modeling results can suggest mechanisms underlying differences between drugs or drug classes.

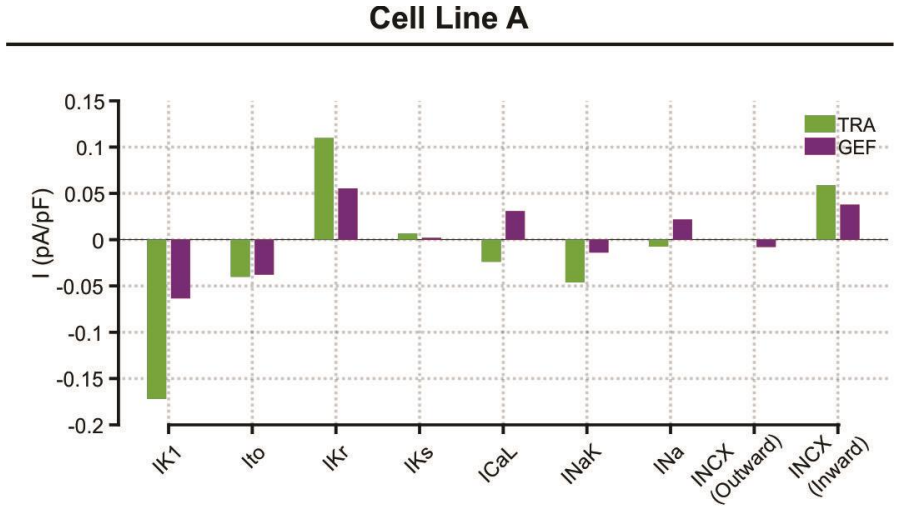
Mechanisms underlying arrhythmia susceptibility

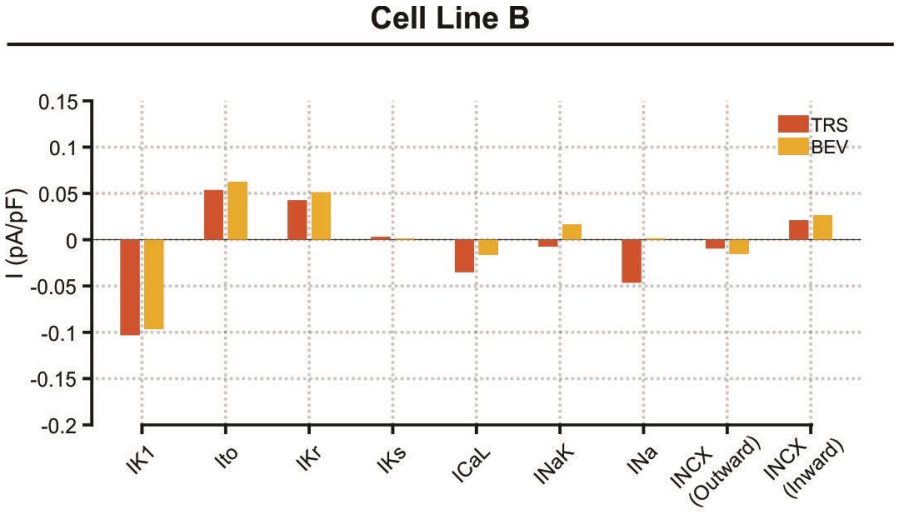
Simulate control and TKI-treated cells at reduced [K^+]

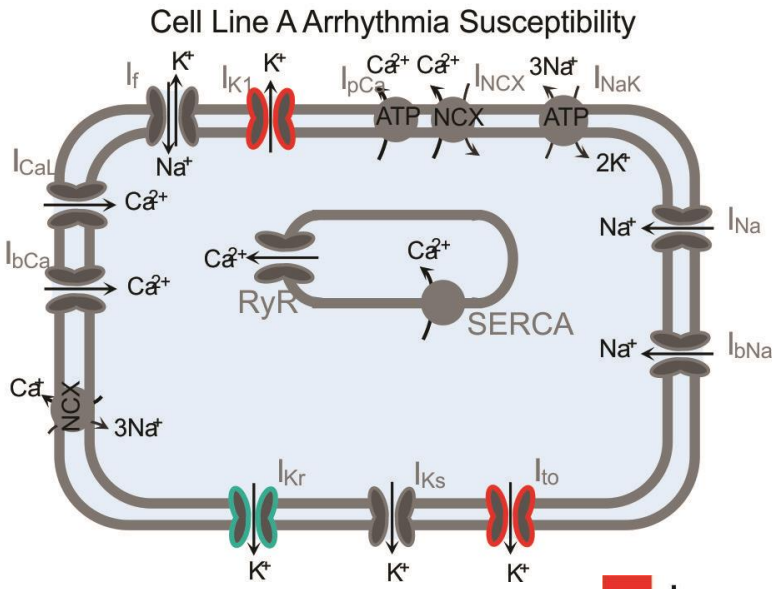
Compute change in total charge (integrated current) through each ion channel

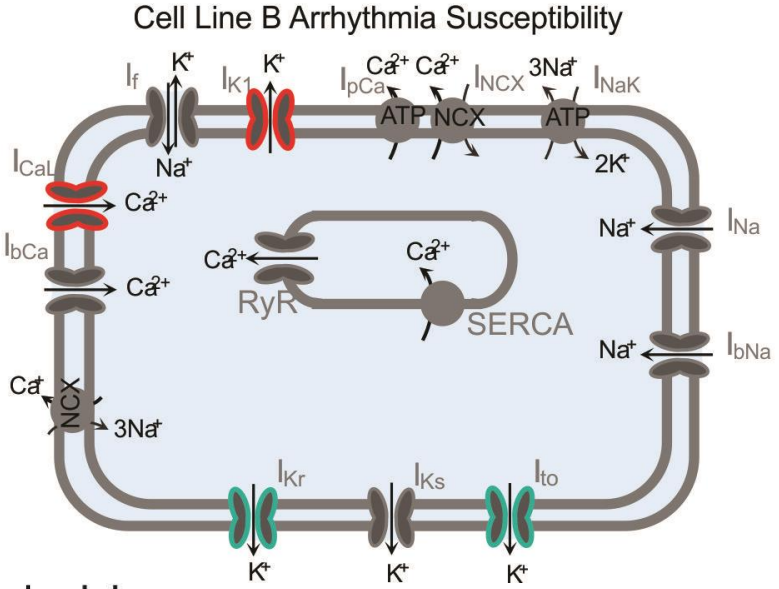


Mechanisms underlying arrhythmia susceptibility







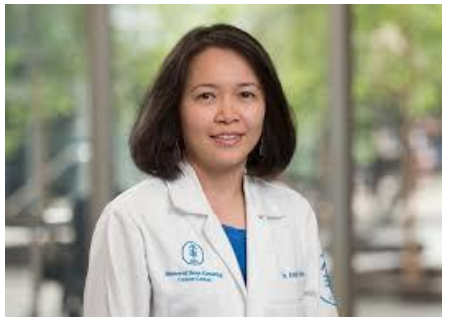


Increase proarrhythmic risk

decrease proarrhythmic risk

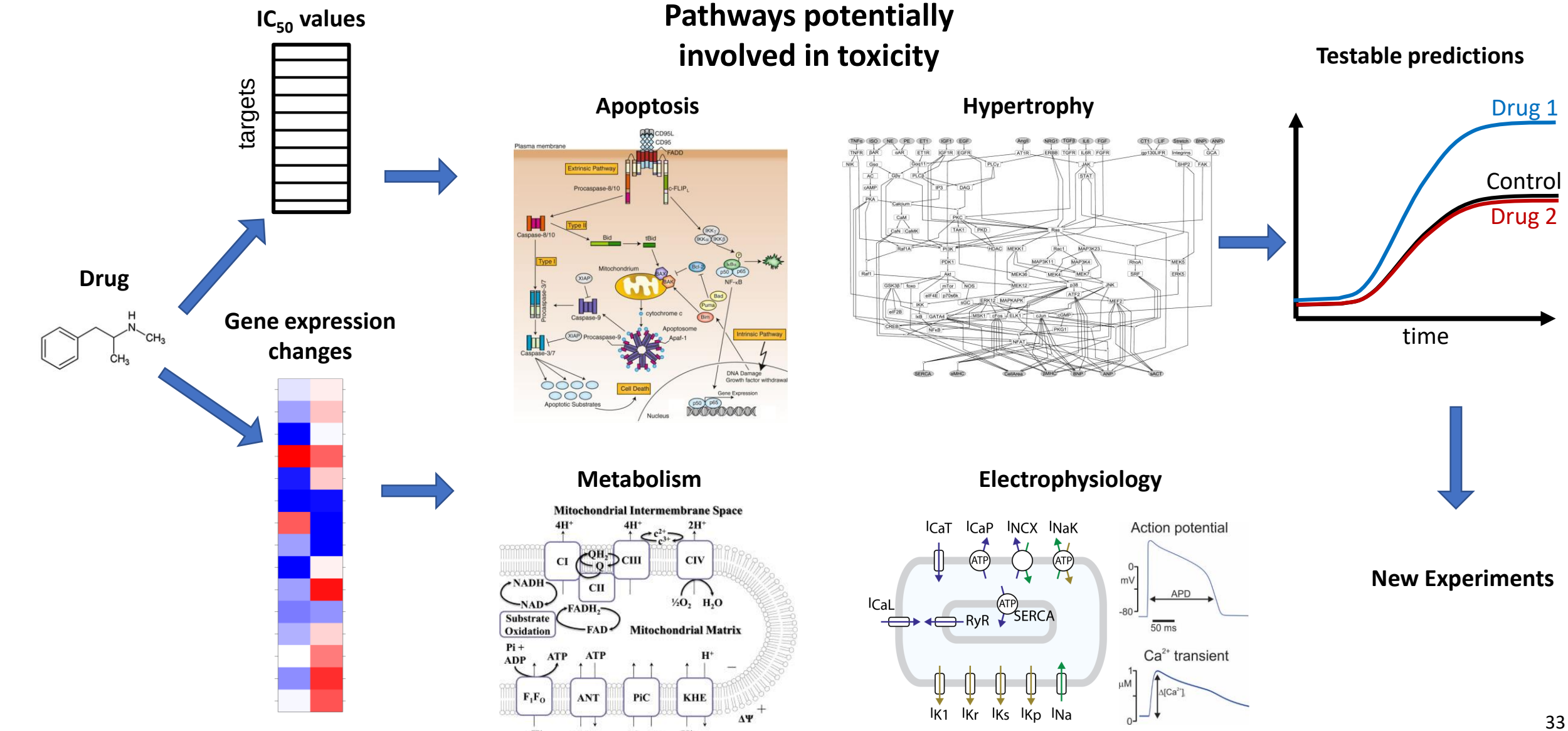
Future Directions: further testing these hypotheses

- Validate the changes in ionic currents that are predicted to be critical to altered arrhythmia susceptibility
- More cell lines from healthy volunteers. Is there something unusual about one of the two that we tested?
- Correlate iPSC-CM susceptibility with clinical outcomes
  - Collaboration with Angel Chan, Memorial Sloan Kettering
  - Patients who developed trastuzumab cardiotoxicity



- Expand the mathematical modeling pipeline to incorporate additional cardiotoxicity mechanisms

Future Directions: PredicTox Knowledge Environment



Conclusions

Combining mRNAseq data with mechanistic models allows us to address the causes of drug-induced cardiotoxicity

Simulations allow us to rank drugs within a class, compare drug classes, and prioritize physiological experiments

Results suggest that short-term treatment with TKIs does not induce overt cardiotoxicity, but can influence susceptibility to physiological stimuli

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Learn and Live

NIH LINCS PROGRAM