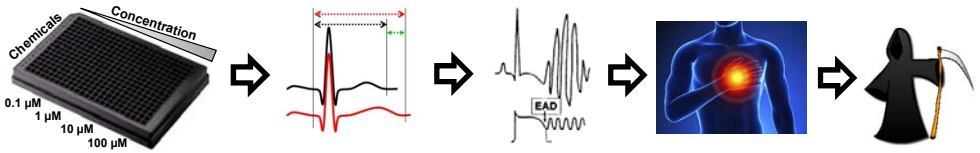
Development and Use of Quantitative Adverse Outcome Pathways:

Lessons Learned from Application to Cardiotoxicity



Weihsueh A. Chiu, PhD
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On behalf of the TAMU/NCSU EPA STAR Center

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Outline

- Background – Cardiotoxicity of xenobiotics
- qAOP for QT/QTc prolongation as a case study integrating *in vitro*, *in silico*, and *clinical* data
- Lessons learned

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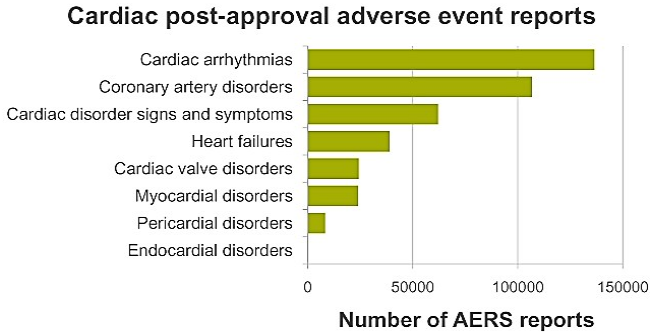
Thorough QT/QTc in a Dish: An In Vitro Human Model That Accurately Predicts Clinical Concentration-QTc Relationships.
Blanchette AD, Grimm FA, Dalaijamts C, Hsieh NH, Ferguson K, Luo YS, Rusyn I, **Chiu WA**. Clin Pharmacol Ther. 2018 Oct 22. doi: 10.1002/cpt.1259. [Epub ahead of print] PMID: 30346629

Cardiotoxicity Hazards of Xenobiotics

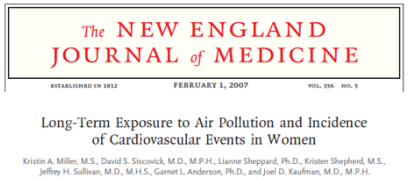
Pharmaceuticals: *YES*

Phase	Non-clinical	Phase I	Phase I-III	Phase III/ post-approval	Post-approval	Post-approval	Post-approval
Information	Causes of attrition	Serious ADRs	Causes of attrition	ADRs on label	Serious ADRs	Withdrawal from sale	Withdrawal from sale
Source	Car (2006)	Sibille et al. (1998)	Olson et al. (2000)	BioPrint® (2006)	Budnitz et al. (2006)	Fung et al., (2001)	Stevens & Baker (2009)
Sample size	88 CDs stopped	1,015 subjects	82 CDs stopped	1,138 drugs	21,298 patients	121 drugs	47 drugs
Cardiovascular	27%	9%	21%	38%	15%	9%	45%
Hepatotoxicity	8%	7%	21%	13%	0%	25%	32%



Environmental Exposures: *??*

- **Air Pollution: *YES***


The NEW ENGLAND JOURNAL of MEDICINE
ESTABLISHED IN 1812 FEBRUARY 1, 2007 VOL. 356 NO. 5

Long-Term Exposure to Air Pollution and Incidence of Cardiovascular Events in Women

Kristin A. Miller, M.S., David S. Siscovick, M.D., M.P.H., Lianne Sheppard, Ph.D., Kristen Shepherd, M.S., Jeffrey H. Sullivan, M.D., M.H.S., Garnet L. Anderson, Ph.D., and Joel D. Kaufman, M.D., M.P.H.

Published in final edited form as:
Epidemiology. 2016 March ; 27(2): 284–290. doi:10.1097/EDE.0000000000000424.

Association between Particulate Air Pollution and QT Interval Duration in an Elderly Cohort

Irina Mordukhovich[1], Itai Kloog[1,2], Brent Coull[3], Petros Koutrakis[1], Pantel Vokonas[4], and Joel Schwartz[1,5]

- **Other exposures: *Maybe***
  - *Little data beyond epidemiologic studies of a few chemicals (air pollution, lead, environmental tobacco smoke,…)*
  - *Not routinely tested for in experimental animal studies*

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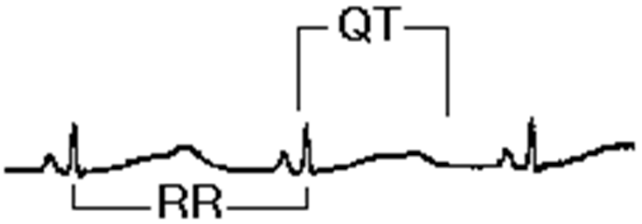
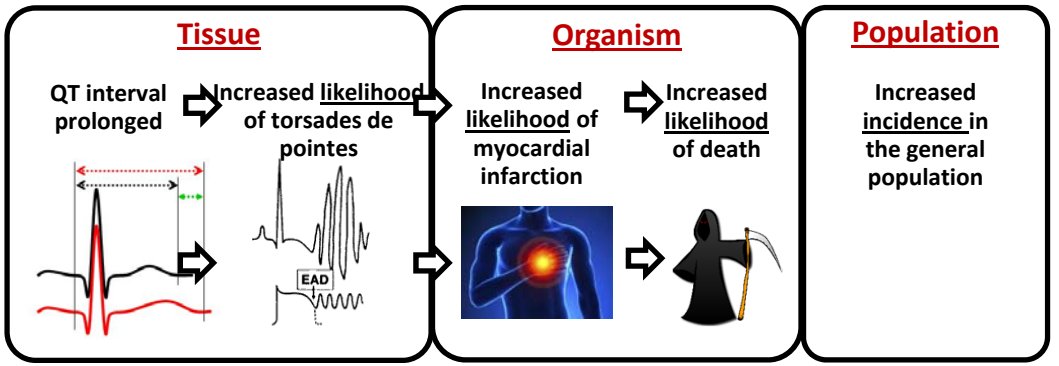
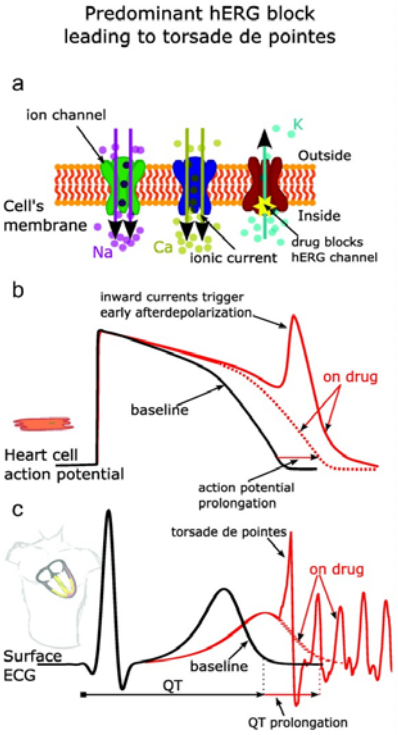
QT interval at a biomarker of cardiovascular risk

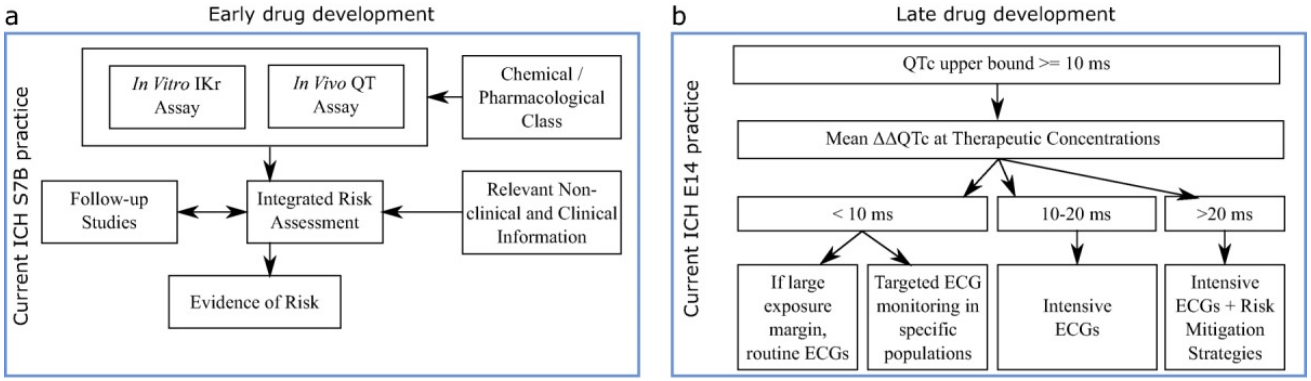
- Genetic and drug-induced QT prolongation known to increase risk of sudden cardiac death.
- Emerging (last 3-5 years) literature on baseline QT as a risk factor in the general population:
  - Sudden cardiac death (e.g., Deo et al. 2016);
  - Major cardiovascular event or death (e.g., Shah et al. 2016);
  - Stroke, independent of atrial fibrillation (e.g., O'Neal et al. 2015).





Current Drug Testing Strategy for Cardiotoxicity Focuses on QT prolongation





- Multi-million dollar clinical trial – the "Thorough QT/QTc" (TQT) study – required even without preclinical concerns.
- Threshold of regulatory concern = "upper bound of the 95% confidence interval around the mean effect on QTc of 10 ms."
- Highly successful in reducing cardiotoxicity in approved drugs.

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Source: Vicente et al., 2018. Mechanistic Model-Informed Proarrhythmic Risk Assessment of Drugs: Review of the "CiPA" Initiative and Design of a Prospective Clinical Validation Study, Volume: 103, Issue: 1, Pages: 54-66, First published: 07 October 2017, DOI: (10.1002/cpt.896)

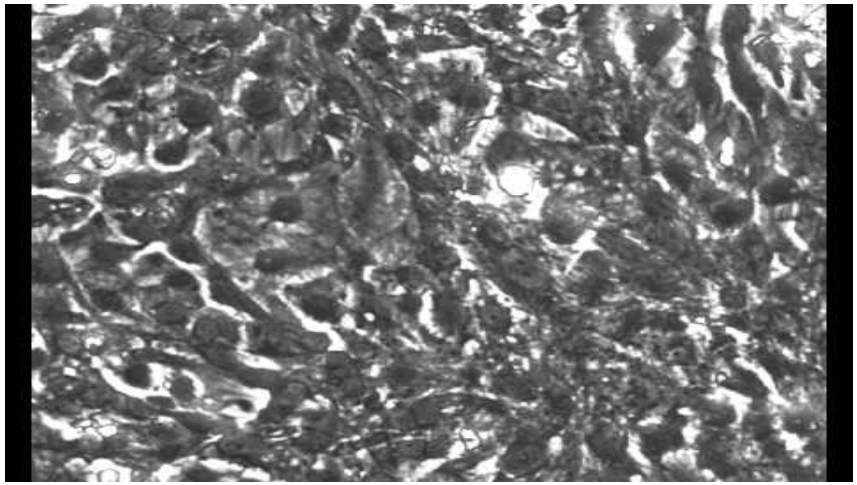
Current Chemical Safety Testing Strategy for Cardiotoxicity … *Does Not Exist*

- Rodents are fed low fat diets, and are not monitored for cardiotoxicity beyond pathology.
- Main preclinical models (e.g., dog) are not routinely used for non-pharmaceuticals.
- Most data on cardiovascular effects of chemicals is from epidemiology – effects may already be occurring in the population.
- *How can mechanistic data help inform cardiotoxicity?*

Current Chemical Safety Testing Strategy for Cardiotoxicity … *Does Not Exist*

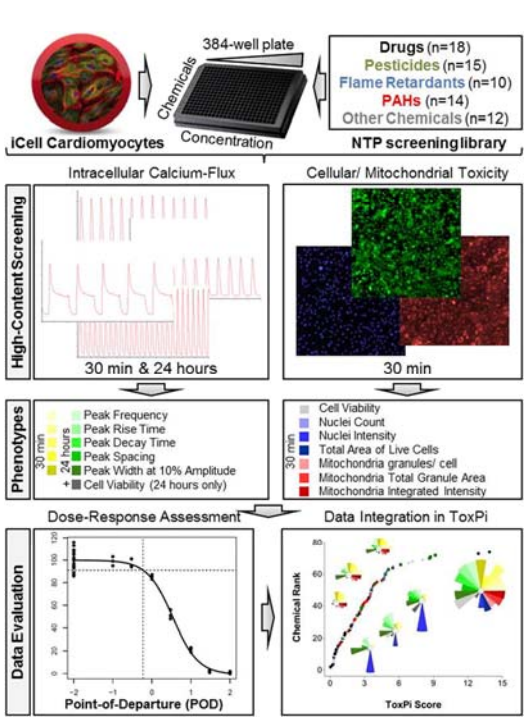
Human Epidemiology	Experimental Animals	Mechanistic Data
• Data available only for a few well-studied chemicals	• Rodents are fed low fat diets, and are not monitored for cardiotoxicity beyond pathology. • Main preclinical models (e.g., dog) are not routinely used for non-pharmaceuticals.	• In vitro channel blocking assays are not routinely used for non-pharmaceuticals. • **iPSC-derived cardiomyocytes**

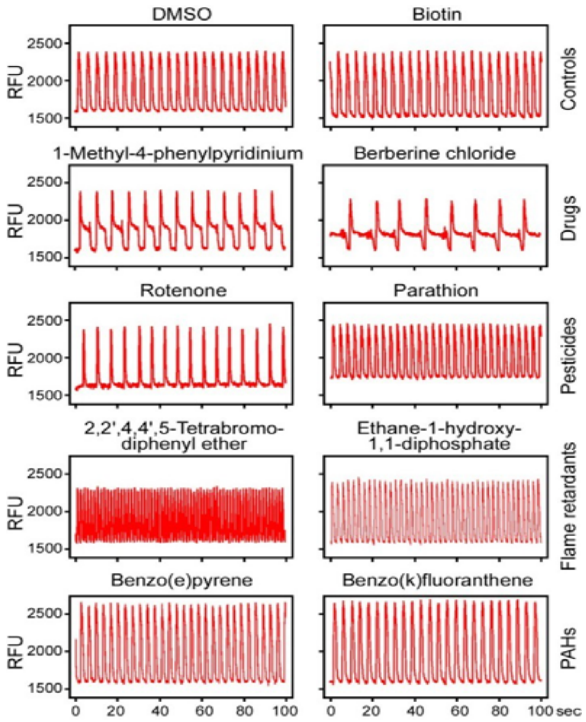
Potential of iPSC-derived cardiomyocytes



- Low cost (compared to clinical trials)
- Potential for population-level and patient-specific testing
- Phenotypically-relevant
  - Beat synchronously *in vitro*
  - Exhibit expected response to congenital and drug-induced cardiotoxicity
- Reproducible resource (unlike primary cells)

Proof of Principle Recently Published





Potential Cardiotoxicity from Environmental Chemicals?

- Cardiomyocytes *in vitro* reproduce *in vivo* phenotypes for cardiotoxic drugs.
- Environmental chemicals also affect beating rhythm and other parameters *in vitro.*

Sirenko O, Grimm FA, Ryan KR, Iwata Y, Chiu WA, Parham F, Wignall JA, Anson B,Cromwell EF, Behl M, Rusyn I, Tice RR. *In vitro* cardiotoxicity assessment of environmental chemicals using an organotypic human induced pluripotent stem cell-derived model. Toxicol Appl Pharmacol. 2017 May 1;322:60-74. doi:10.1016/j.taap.2017.02.020. Epub 2017 Mar 1. PubMed PMID: 28259702; PubMedCentral PMCID: PMC5734940.

Potential role of (q)AOPs in systematic review of mechanistic data

- Organizing framework for scoping and problem formulation (NASEM 2017 Report; Smith et al. 2016).

- Relating *in vitro* concentrations associated with mechanistic data to evidence on internal and external doses associated with health effects *in vivo* in animals and humans (beyond "standard" IVIVE).

- Relating the evidence from short-term mechanistic studies to outcomes after longer term exposure in animals and humans.

QT/QTc prolongation as a case study

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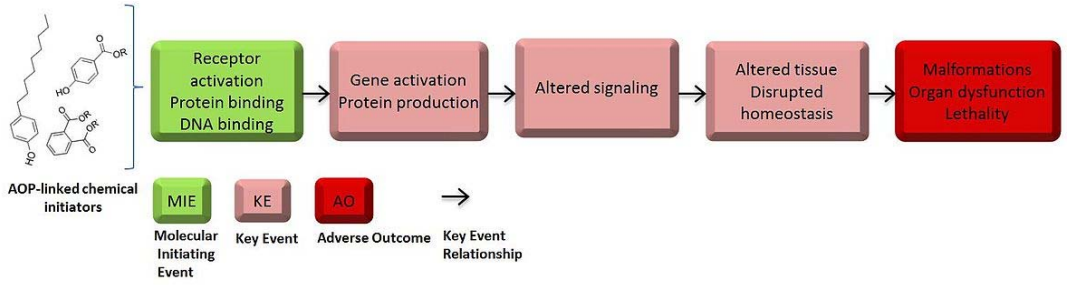
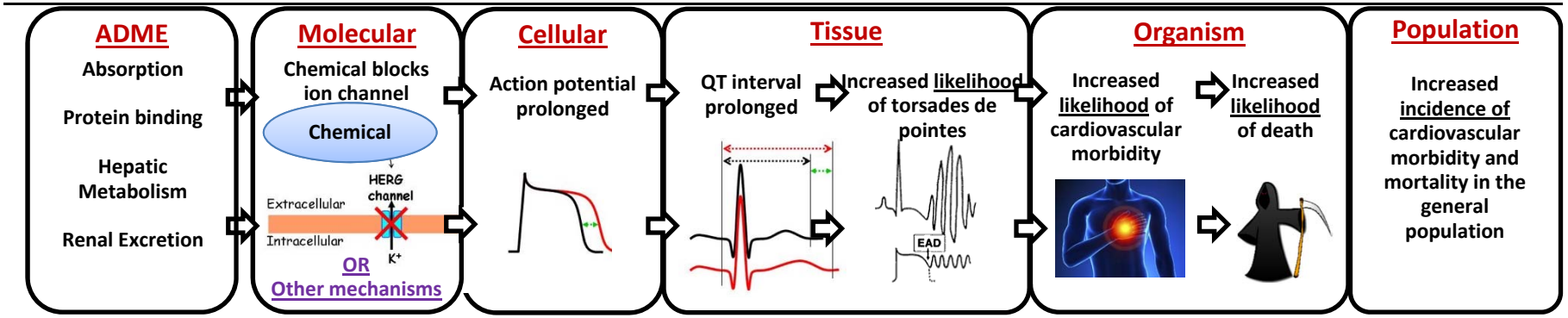


- Quantitative AOP enables incorporation of stochastic events and population variability
- Quantitative AOP predicting *exposure-response*
  - Provides greater confidence in Hazard (*a la* "Hill" criteria/GRADE, etc.)
  - Can also inform dose-response assessment

(q)AOP for QT/QTc prolongation



In vitro-in silico TK model

In vitro-in silico TD model

In vivo effects at *individual* and *population* levels based on clinical and epidemiologic data

Incorporate population variability

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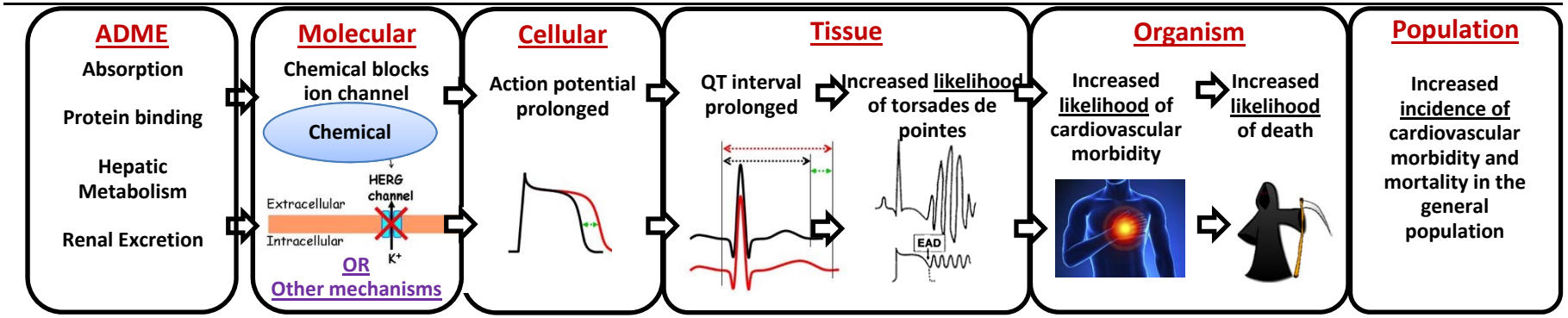
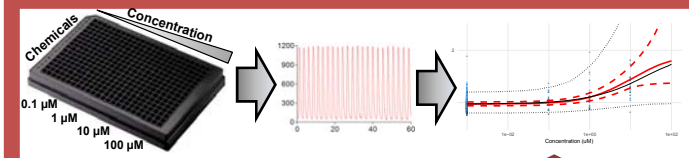
(q)AOP for QT/QTc prolongation



Population-based *in vitro* testing

+

Bayesian concentration-response modeling



Accuracy confirmed by comparing to clinilcal *in vivo* drug data

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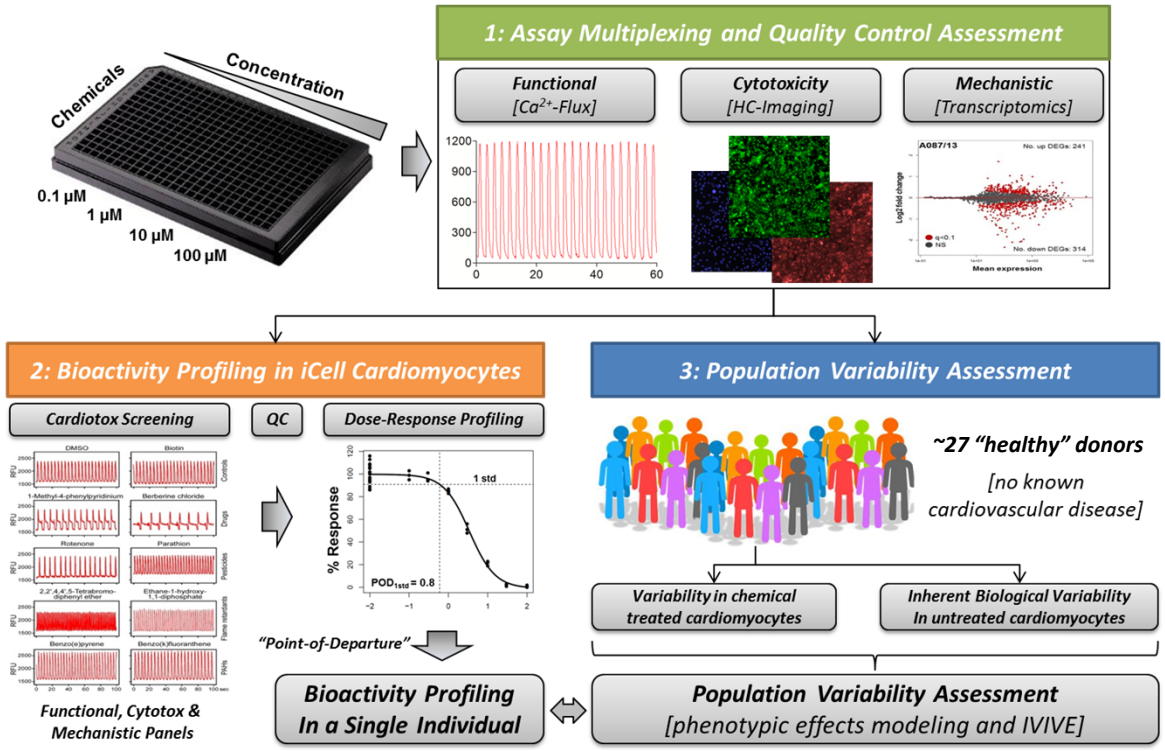
Study Design



Chemicals (drugs) with corresponding *in vivo* clinical data

Positive for *in vivo* QTc prolongation

- Cisapride
- Citalopram
- Disopyramide
- Dofetilide
- Moxifloxacin
- N-acetylprocainamide
- Quinidine sulfate
- Sematilide
- Sotalol
- Vernacalant

Negative for *in vivo* QTc prolongation

- Cabazitaxel
- Lamotrigine
- Mifepristone

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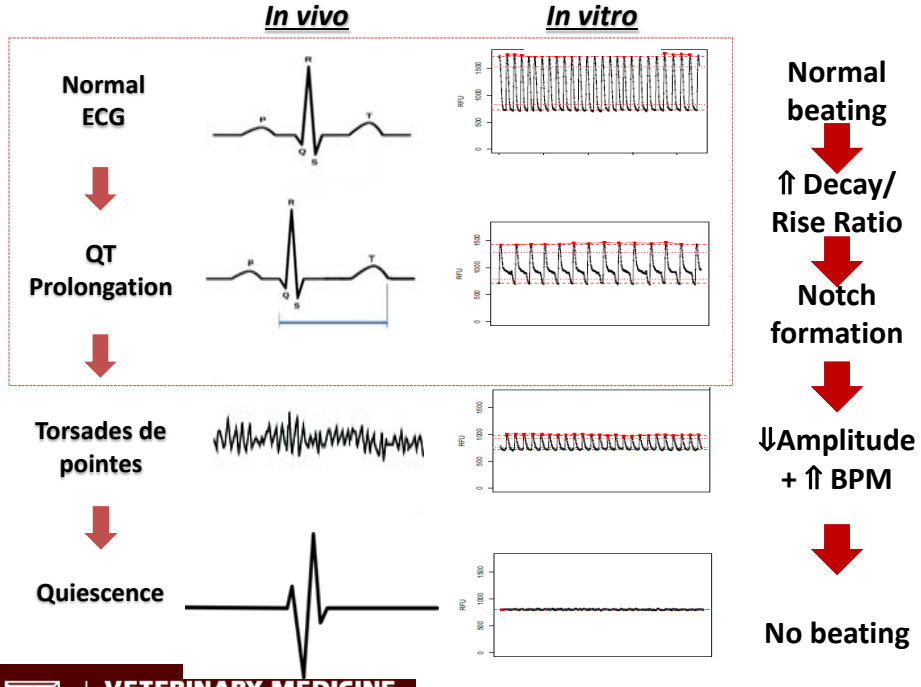
Blanchette et al., 2018; Sirenko et al. 2013, 2017

Establishing qualitative and quantitative *in vivo* and *in vitro* correspondence

Qualitative Comparison



Quantitative Comparison

- ***In vivo*:** use published PD modeling results for concentration-response relationships for QTc
- ***In vitro*:** conduct Bayesian population PD modeling (Chiu et al. 2017) of decay-rise ratio
- **Compare *in vivo* and *in vitro*** concentration-response relationships (e.g., median and their CI)

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Establishing qualitative and quantitative *in vivo* and *in vitro* correspondence

In Vivo

Common dose metric

- Literature-based values for free fraction in serum used to re-scale total concentrations to free concentrations

Common effect metric

- Study-specific values for baseline QTc used to re-scale responses to percent change from baseline

In Vitro

Common dose metric

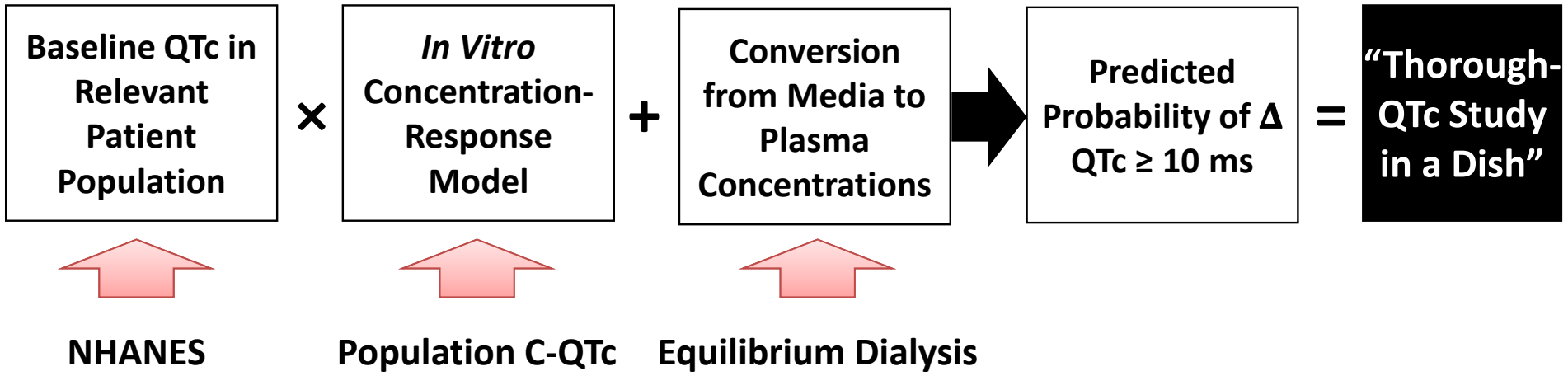
- Free fraction measured in serum and cardiomyocyte media using Rapid Equilibrium Dialysis
- Media free fraction results compared to those from mass-balance model

Common effect metric

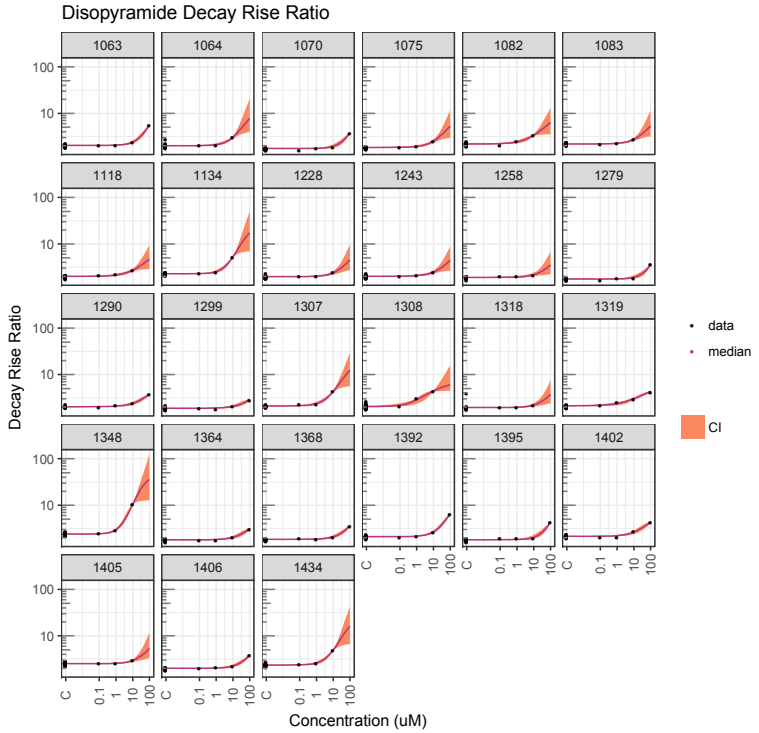
- Reparameterized Hill directly predicts percent change from baseline

Model predictions restricted to concentrations ≤ study-specific Cmax.

Study Design:
Clinical Translation



Results:
Model Development and Evaluation



- All 10 positive control drugs exhibited
  - Increased decay-rise ratio in multiple donors
  - Notch formation in multiple donors
- For 3 negative control drugs
  - Some donors exhibited increased decay-rise ratio
  - No donors exhibited notch formation
- Population concentration-response model accurately fit experimental data

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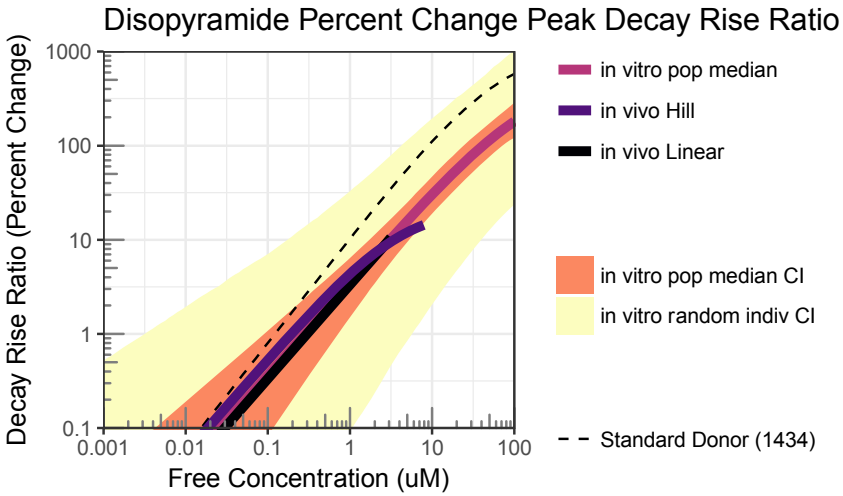
Results:
Qualitative Predictions (Hazard)

- ***In vivo hazard* for QTc prolongation** can be predicted from *in vitro* data

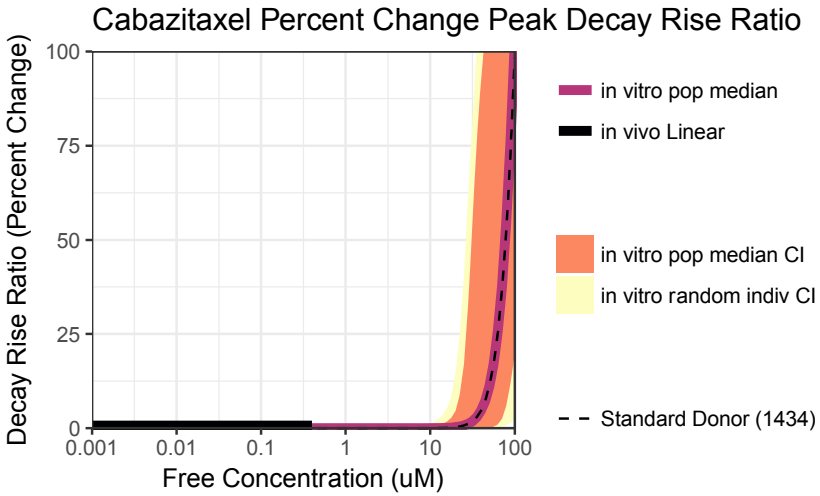
- *In vitro* model correctly predicted observed effect/no effect at *in vivo* free Cmax
  - Known positive compounds:
    Predicted effects from 1% to 46% at *in vivo* free Cmax
  - Known negative compounds:
    Predicted effects < 0.01% at *in vivo* free Cmax
    Upper confidence bound estimates of <0.5%

Results:
Quantitative Predictions

Positive Control



Negative Control



Highly Consistent Concentration-Response Relationships *in vitro* to *in vivo*!

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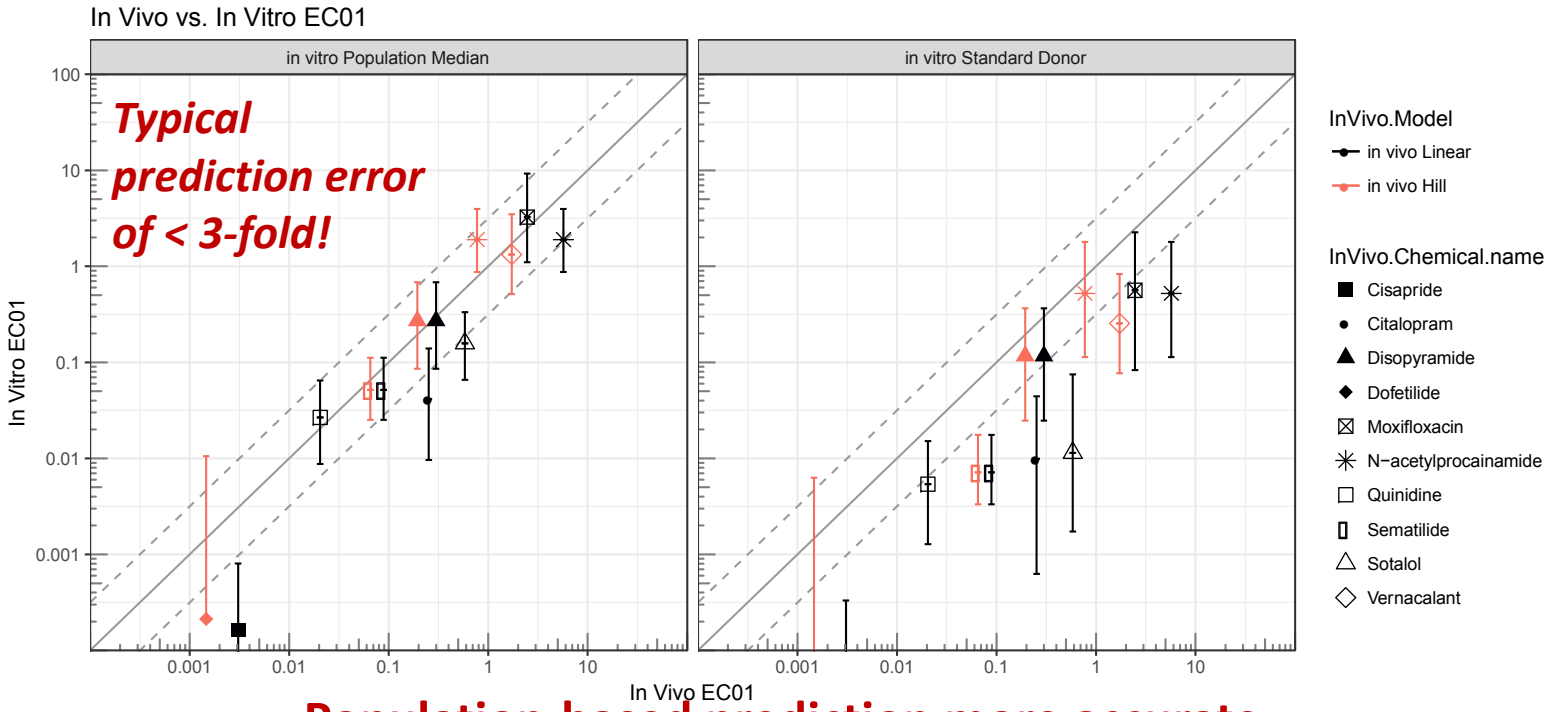
Results:
Quantitative Predictions



Population-based prediction more accurate and more precise than using a single donor

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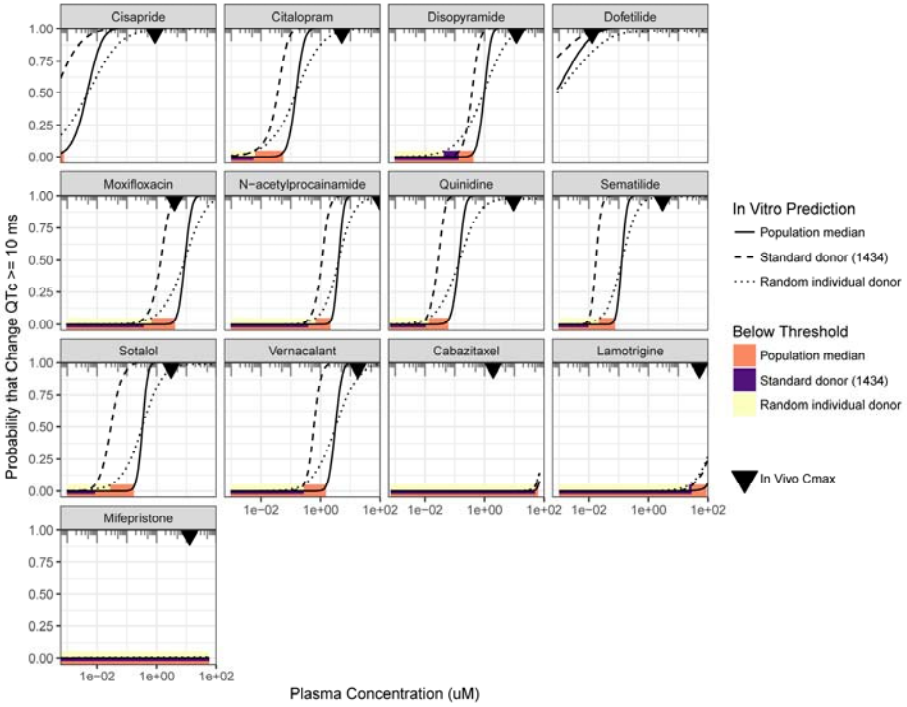
Results:
Clinical Translation



- Clinical translation of *in vitro* C-QTc modeling results involves determining the probability that clinical $\Delta QTc(x_{plasma})>10$ ms
- All the positive controls except moxifloxacin, clearly fail the regulatory safety threshold at C_{max}.
- All negative controls except lamotrigine clearly satisfy the regulatory safety threshold.
- For moxifloxacin and lamotrigine, results more ambiguous, with different conclusions at population versus individual level (consistent with clinical literature).

Summary of Results

The combination of a population-based *in vitro* model and *in silico* pharmacodynamic modeling can accurately predict the results of the *in vivo* clinical TQT study:

- Concentration-QTc relationship
- Range of clinical concentrations that satisfy the regulatory threshold (<10 msec at 95% confidence)

Why quantitative AOP?

- Most AOPs present a deterministic series of events in homogenous populations
- Most AOPs can only inform Hazard ID

Quantitative AOP enables incorporation of stochastic events and population variability

Quantitative AOP predicting *exposure-response*

- Provides greater confidence in Hazard (*a la* "Hill" criteria/GRADE, etc.)
- Can also inform dose-response assessment

(q)AOP for QT/QTc prolongation

ADME

Absorption

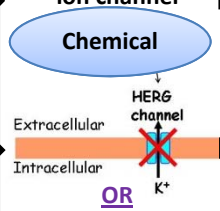
Protein binding

Hepatic Metabolism

Renal Excretion

Molecular

Chemical blocks ion channel

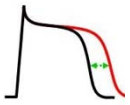


OR

Other mechanisms

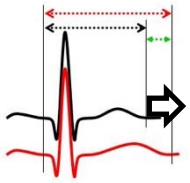
Cellular

Action potential prolonged

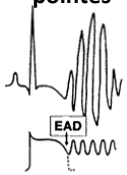


Tissue

QT interval prolonged



Increased likelihood of torsades de pointes



Organism

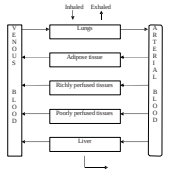
Increased likelihood of cardiovascular morbidity

Increased likelihood of death

Population

Increased incidence of cardiovascular morbidity and mortality in the general population

Population RTK (IVIVE) Models



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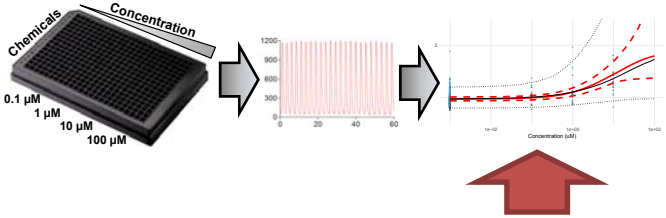
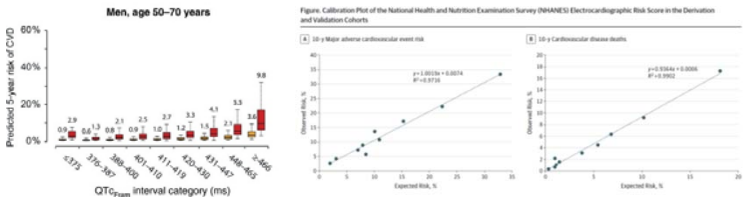


Accuracy confirmed by comparing to clinilcal *in vivo* drug data

Published Clinical Epidemiology-Based Cardiovascular Risk Models



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- Relating the evidence from short-term mechanistic studies to outcomes after longer term exposure in animals and humans.

Why does it work?

Building a better AOP with biomarkers

Molecular initiating event (MIE) is *not necessary or sufficient* to build a qAOP

Quantitative/predictive biomarker such as QTc can serve as the critical link from *cell/tissue → individual → population*

Advantages:

- Enables evaluation of qualitative and quantitative correspondence between model system (e.g., iPSC-based organotypic cultures) and *in vivo* human effects
- Takes advantage of clinical biomedical literature on biomarkers and risk prediction
- Naturally focuses efforts on endpoints with human relevance and public health impact