# Disclaimer

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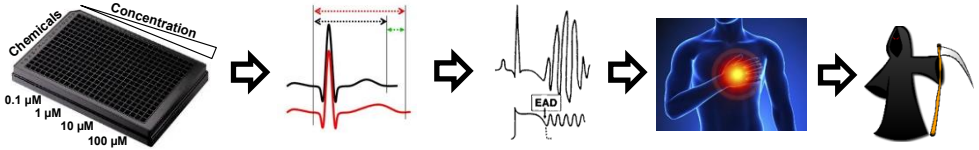
# Microphysiological systems that fill data gaps in human health assessments




**Ivan Rusyn, MD, PhD**
Department of Veterinary Integrative Biosciences
Texas A&M University

# Acknowledgements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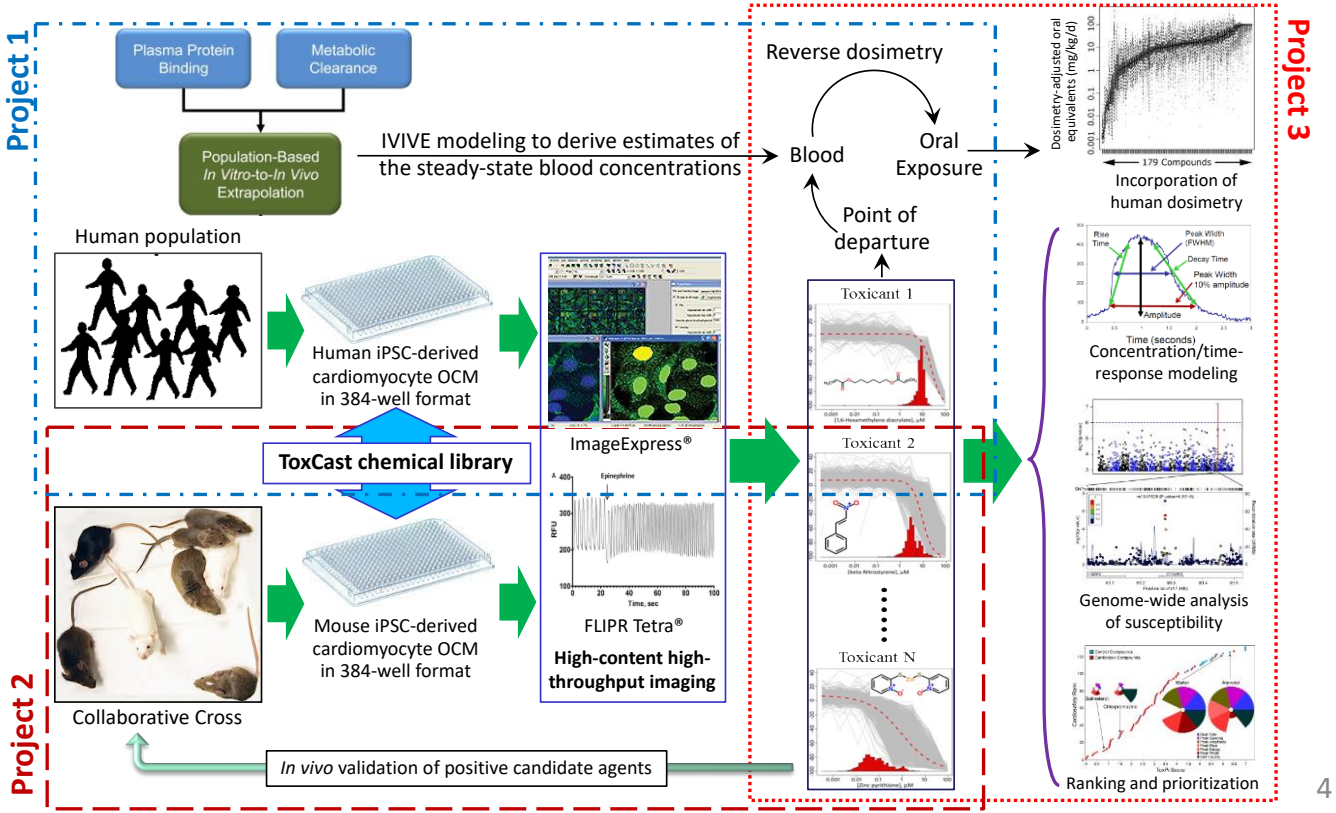
VETERINARY MEDICINE & BIOMEDICAL SCIENCES
TEXAS A&M UNIVERSITY

# Relevance of this program to the EPA

- Established **a model** for testing potential cardiotoxicity of environmental chemicals (**none exists** now even in ToxCast)
- Showed that this human *in vitro* model is physiological, human relevant, reproducible, and **high-throughput**
- Demonstrated that this model can be used to quantify **population variability** in responses to chemicals
- Showed how this *in vitro-in silico* model can make **clinically-relevant predictions for chemical effects** on the heart rhythm

# EPA STAR Center [TAMU-NCSU]
# Organotypic Culture Model Center for **Cardiotoxicity**

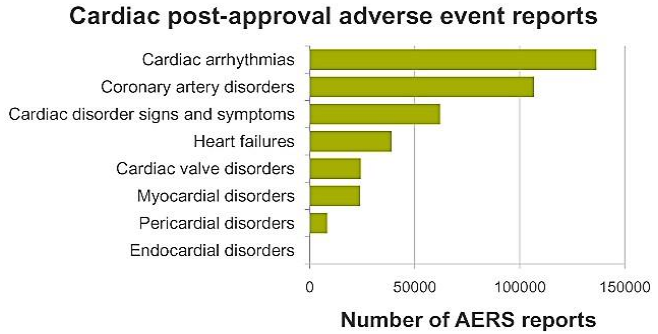
The ***long-term objective*** of the Center is **to *advance regulatory decision-making* *by establishing and validating effective, accurate and fiscally responsible means to identify and characterize cardiac hazards* *from chemical exposures***.



# **Cardiotoxicity** Hazards of Chemicals

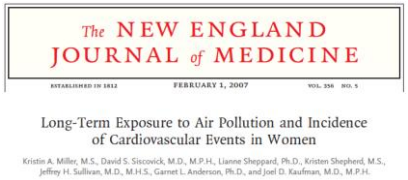
## Pharmaceuticals: *YES*

| 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% | 36% | 15% | 9% | 45% |
| Hepatotoxicity | 8% | 7% | 21% | 13% | 0% | 36% | 23% |




## Environmental Chemicals: *??*

- **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.

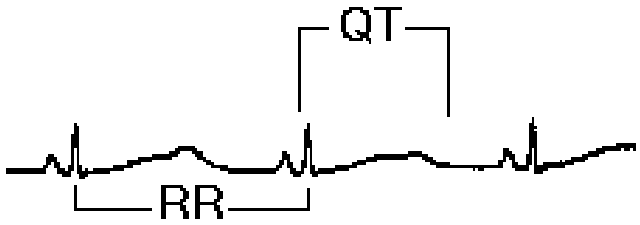
**Association between Particulate Air Pollution and QT Interval Duration in an Elderly Cohort**

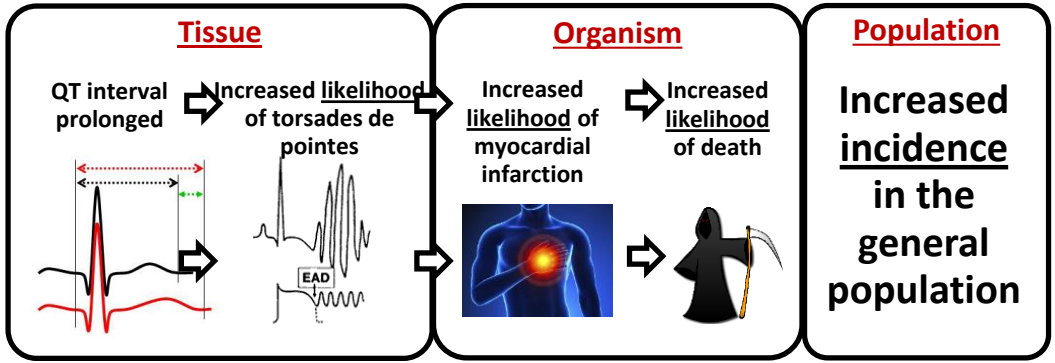
Irina Mordukhovich[1], Itai Kloog[1,2], Brent Coull[3], Petros Koutrakis[1], Pantel Vokonas[4], and Joel Schwartz[1,5]

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

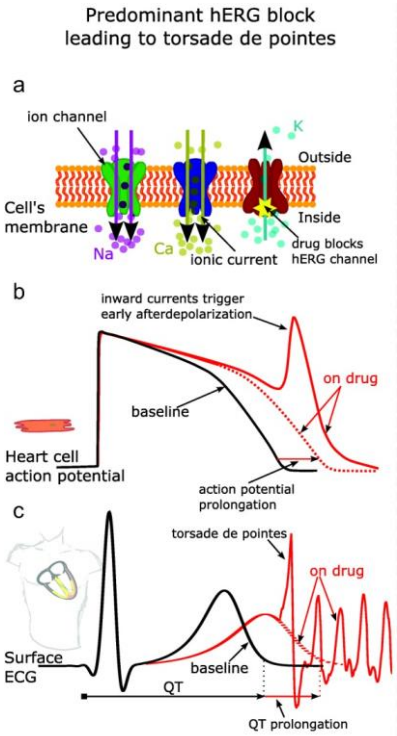
# QT interval as a biomarker of cardiac disease risk

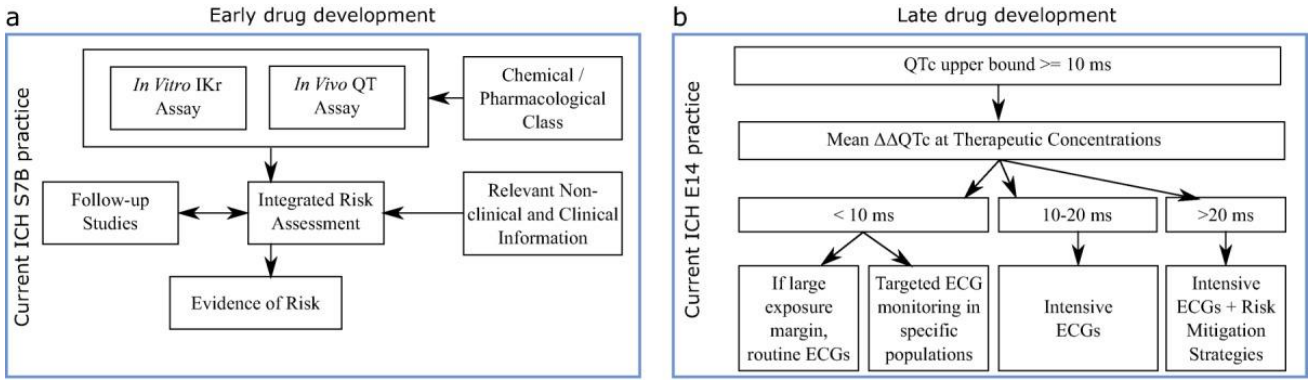
- Genetic and drug-induced QT prolongation known to increase risk of sudden cardiac death.
- Emerging (last 3-5 years) literature on baseline QT as a risk factor in the general population:
  - Sudden cardiac death (e.g., Deo et al. 2016);
  - Major cardiovascular event or death (e.g., Shah et al. 2016);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



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?*

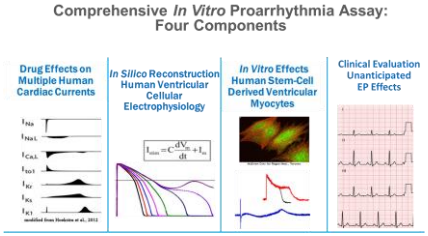
# Limitations of Current Approach

- **High sensitivity, but questions raised about specificity**
- **High cost**
- **Uncharacterized population variability in susceptibility**
- **Cannot conduct clinical trials for non-pharmaceuticals**

CiPA

Cardiac Safety Research Consortium
www.cardiac-safety.org

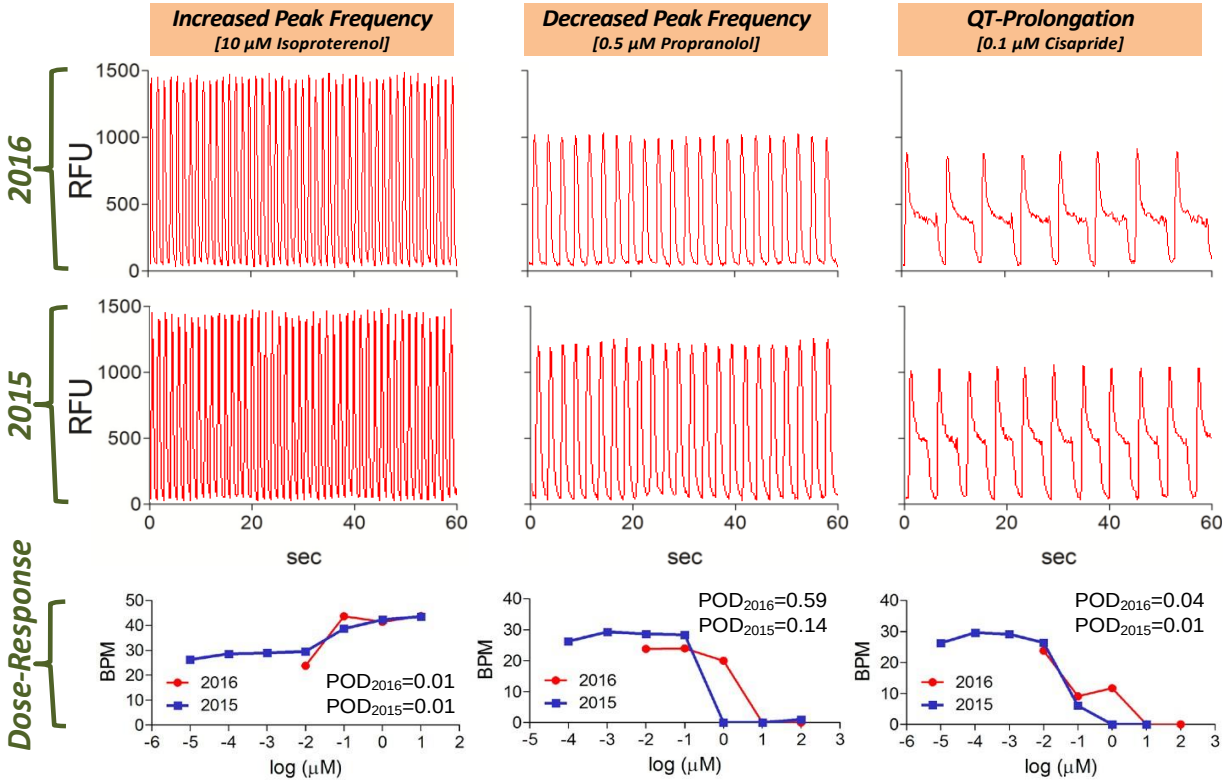
FDA




**Challenges addressed in our Center**

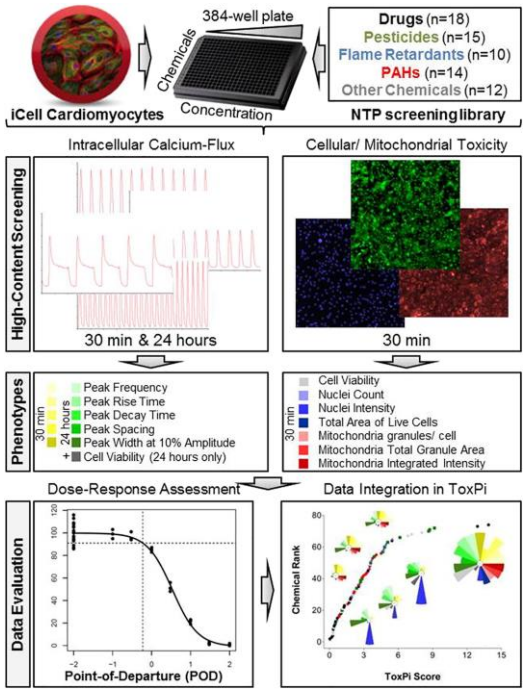
# “Standard” iPSC-derived cardiomyocyte donor is now a well-established model

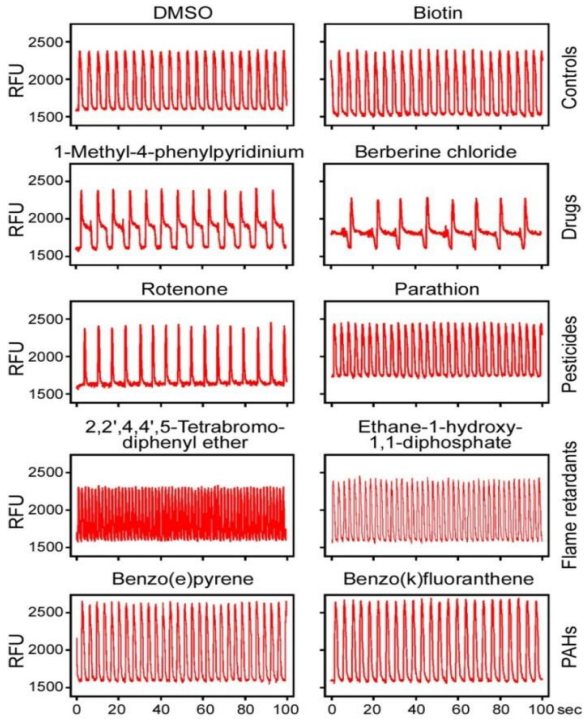
- Treatment-related effects with positive controls are highly reproducible
- **What about chemicals beyond the “positive” controls?**
- **What about cells beyond the “standard donor”?**

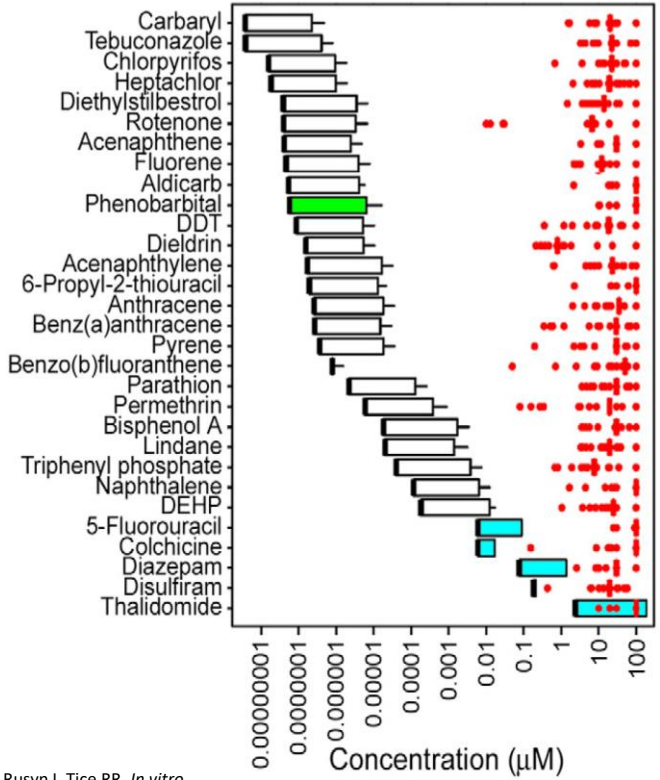



Sirenko et al. (2013); Grimm et al (2015)

# Proof of principle application to **Chemicals** was recently published



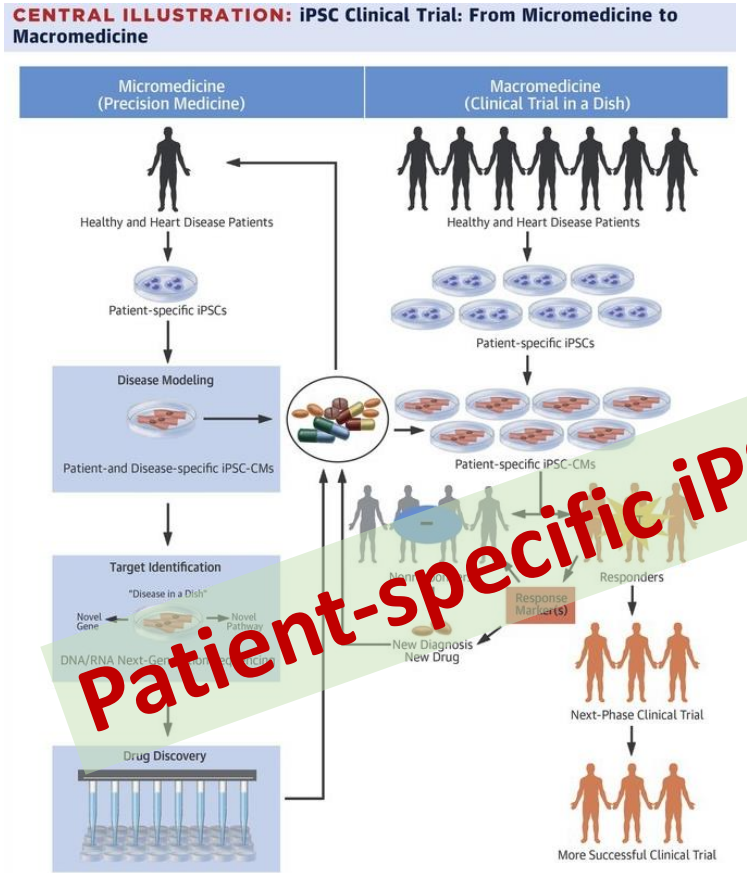





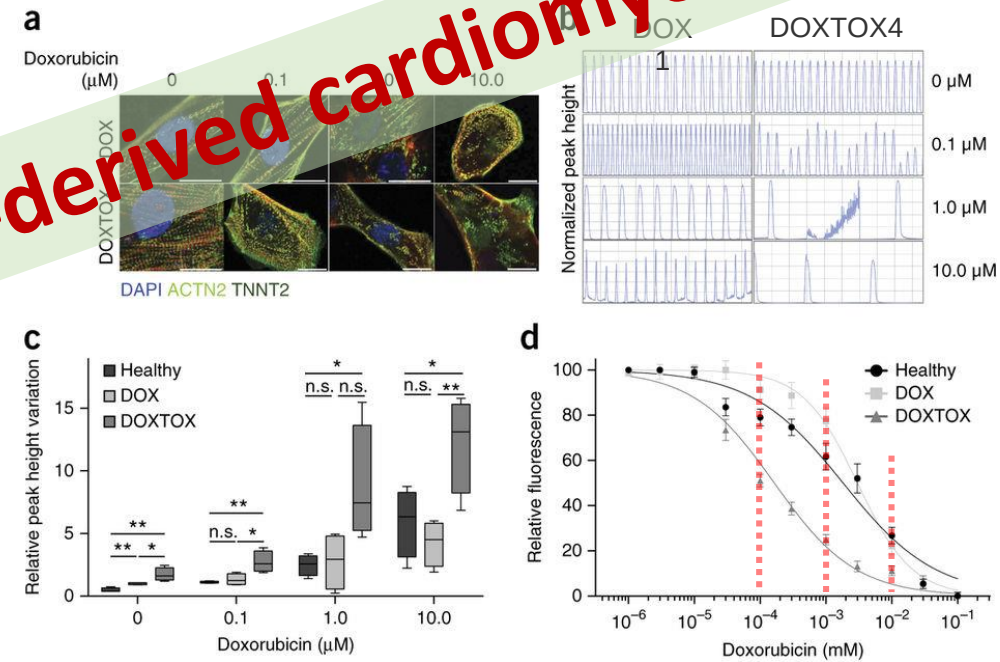

Sirenko O, Grimm FA, Ryan KR, Iwata Y, Chiu WA, Parham F, Wignall JA, Anson B,Cromwell EF, Behl M, Rusyn I, Tice RR. *In vitro* cardiotoxicity assessment of environmental chemicals using an organotypic human induced pluripotent stem cell-derived model. Toxicol Appl Pharmacol. 2017 May 1;322:60-74. PubMed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

# Patient-derived Pluripotent Stem Cells: "*Clinical Trial in a Dish*" and "*Precision Medicine*"




- 12 female subjects used (8 with breast cancer)
  - 4 control volunteers who had never been treated with any chemo drug ('healthy')
  - 4 patients ('DOX') who did not experience clinical cardiotoxicity from doxorubicin
  - 4 patients ('DOXTOX') who did experience clinical cardiotoxicity
- hiPSCs were derived from the skin fibroblasts of each subject and differentiated into patient-derived cardiomyocytes

**Patient-specific iPSC-derived cardiomyocytes**

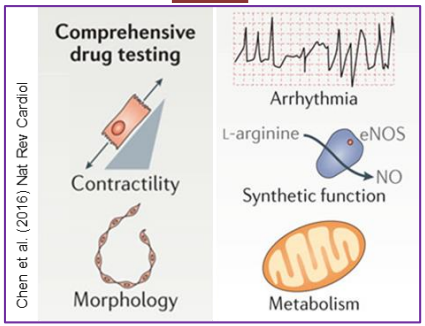



Modified from: Burridge et al. *Nature Medicine* **22**, 547–556 (2016)

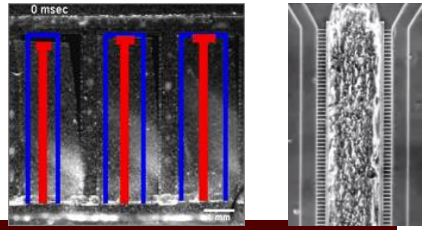
Sayed, N. et al. J Am Coll Cardiol. 2016;67(18):2161-76.

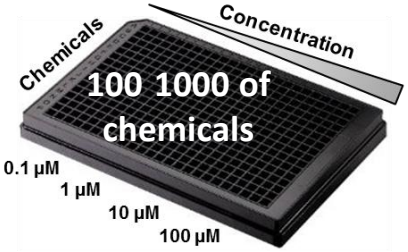
# Organotypic Human *in vitro* Models for Cardiotoxicity Testing

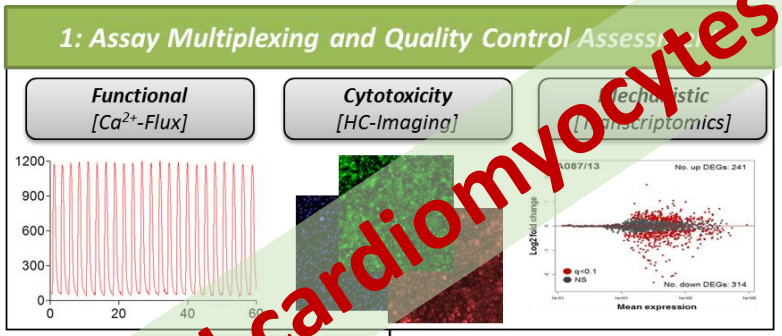
2D

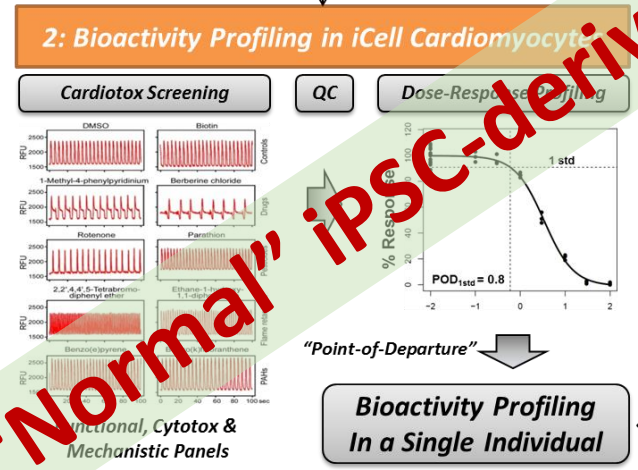


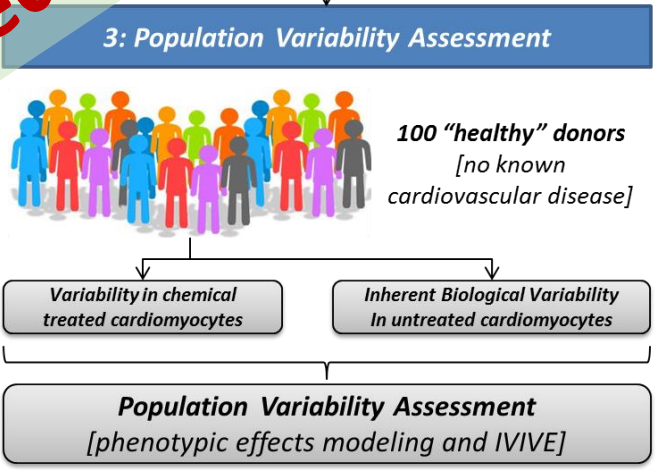

3D



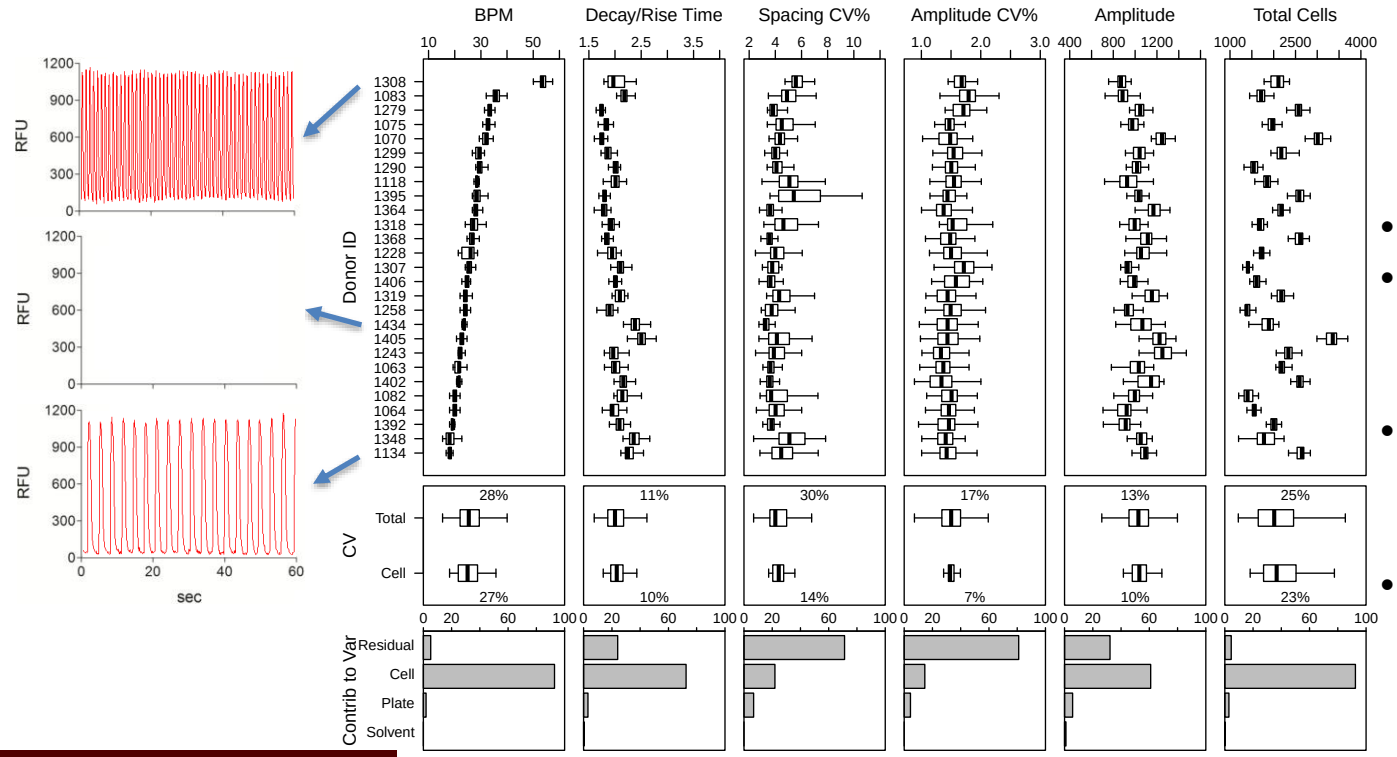



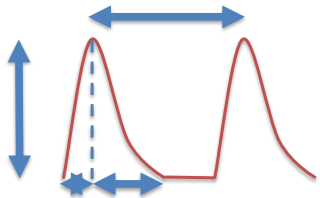

"Normal" iPSC-derived cardiomyocytes

Kitty Parker (Lab
Wyss Institute)
Kevin Healy Lab
U.C. Berkeley)

Sirenko et al Toxicol Appl Pharmacol. 2017; Toxicol Appl Pharmacol. 2013; J Biomol Screen. 2013

# Baseline variability across cell lines is largely ***biological***, not *experimental*

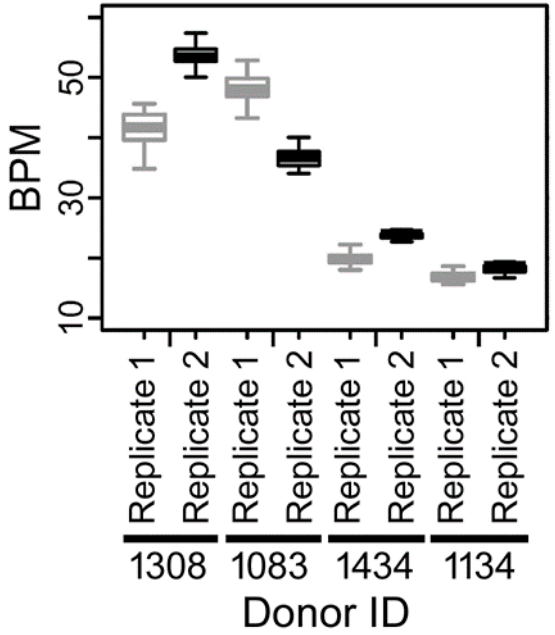


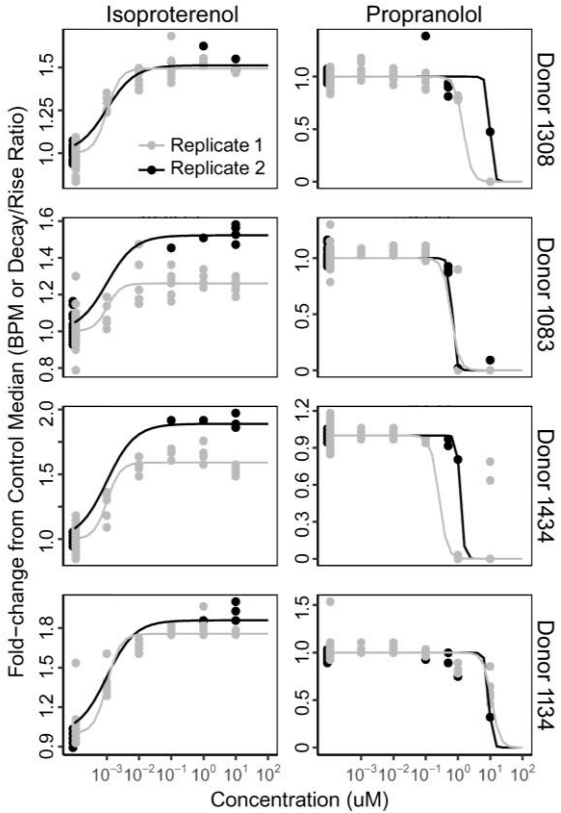



- **BPM** ➔ Heart Rate
- **Decay/Rise Time Ratio** ➔ Surrogate for QTc prolongation
- **Amplitude & Total Cells** ➔ inhibition of beating/cytotoxicity
- **Variation in Peak Spacing or Amplitude** ➔ More severe arrhythmias

Grimm et al., 2018

# Reproducibility of variation



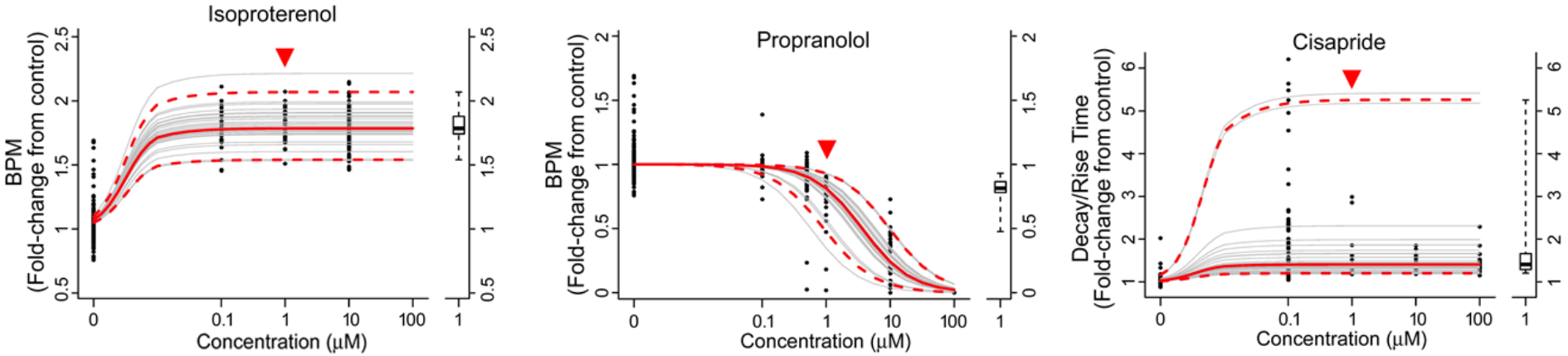




Isoproterenol $E_{max}$ 2016 vs. 2017: $r = 0.80$

Propranolol $\log(EC_{50})$ 2016 vs. 2017: $r = 0.77$

Grimm et al., 2018

# Variability in treatment-related responses is largely ***biological***, not *experimental*

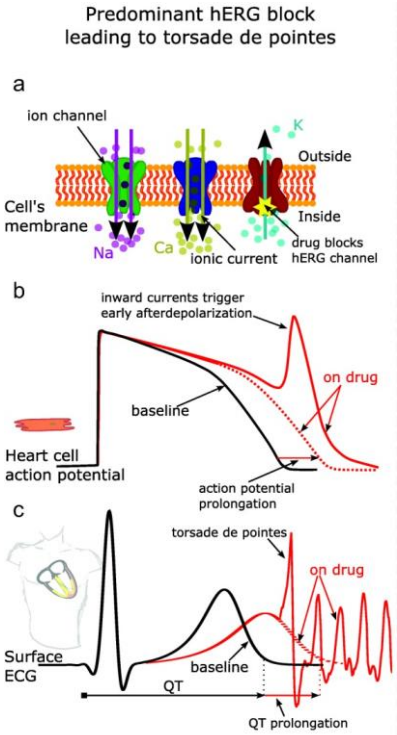


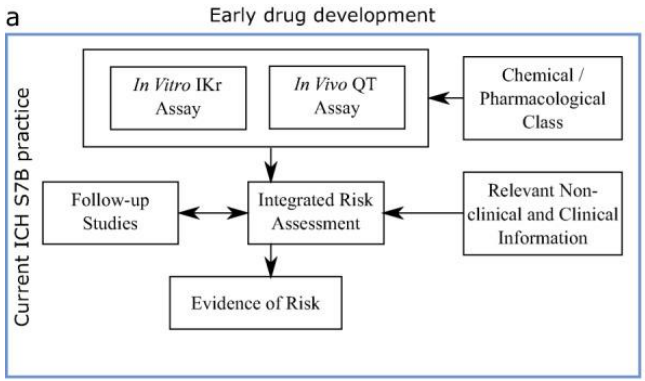

Non-linear random-effects model:
70%-98% of variance is due to inter-individual (donor to donor) variation

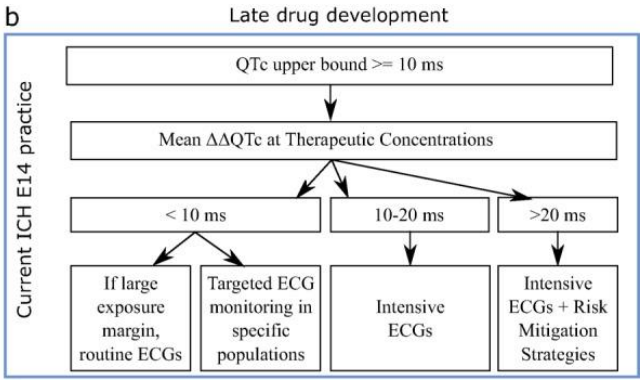


Grimm et al., 2018

# 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 = **5 ms change** → upper bound of the 95% confidence interval around the mean effect on QTc of 10 ms
- Highly successful in reducing cardiotoxicity in approved drugs



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)

# Positive and negative controls with published *in vivo* population PK/PD data and models

**Positive for *in vivo* QTc prolongation**

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

**Negative for *in vivo* QTc prolongation**

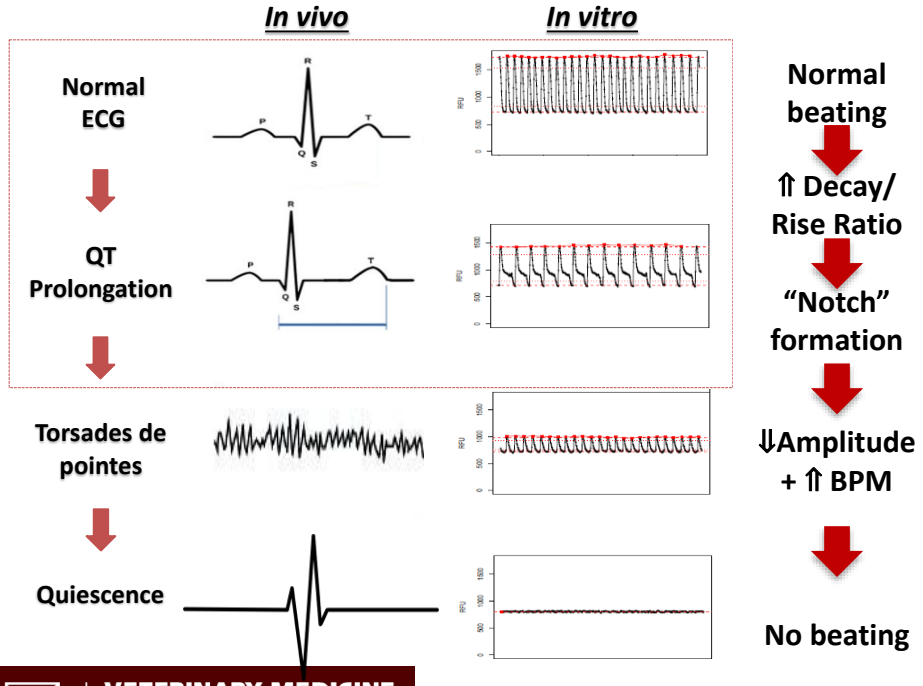
- Cabazitaxel
- Lamotrigine
- Mifepristone

**Simultaneously address (i) Hazard, (ii) Dose-Response, and (iii) Population Variability:**

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

# Establishing qualitative and quantitative *in vivo* to *in vitro* concordance

## *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)

# Establishing qualitative and quantitative *in vivo* to *in vitro* concordance

## *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**

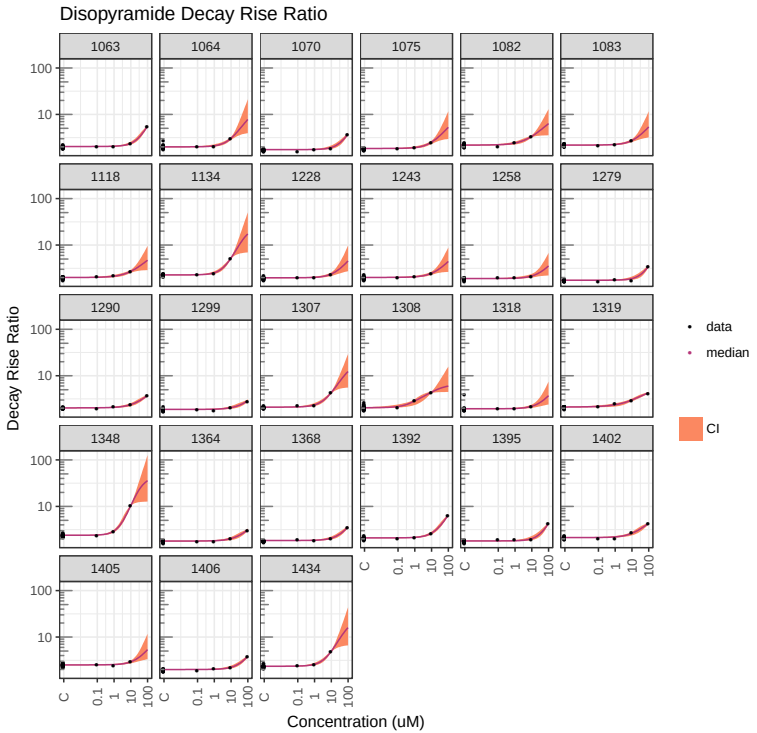
- **Free fraction** measured in serum and cardiomyocyte media using Rapid Equilibrium Dialysis
- Media free fraction results compared to those from mass-balance model

**Common effect metric**

- Re-parameterized Hill directly predicts **percent change from baseline**

***Model predictions restricted to concentrations ≤ study-specific $C_{max}$***

# 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

Blanchette et al., 2019

# Results:
# **Qualitative** Predictions (Hazard)

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

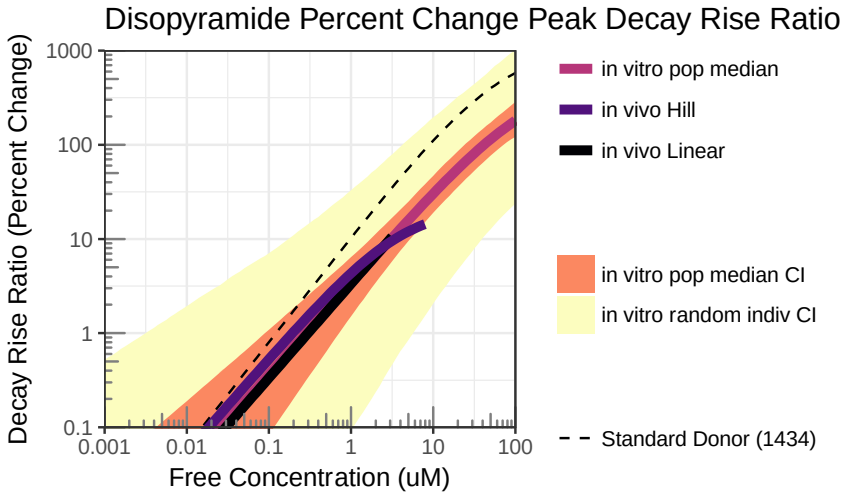
- *In vitro* model correctly predicted observed effect/no effect at *in vivo* free Cmax
  - Known positive compounds:
    Predicted effects from 1% to 46% at *in vivo* free Cmax
  - Known negative compounds:
    Predicted effects < 0.01% at *in vivo* free Cmax
    Upper confidence bound estimates of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



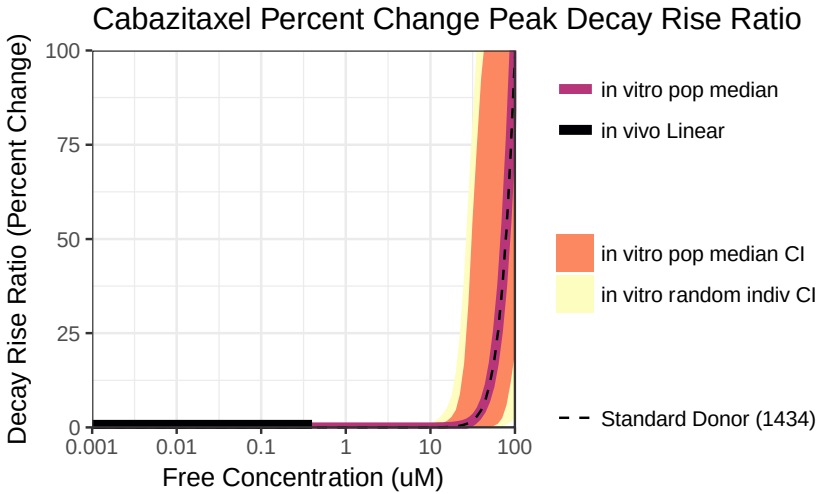
Blanchette et al., 2019

# Results:
# **Quantitative** Predictions (Risk)

## **Positive** Control



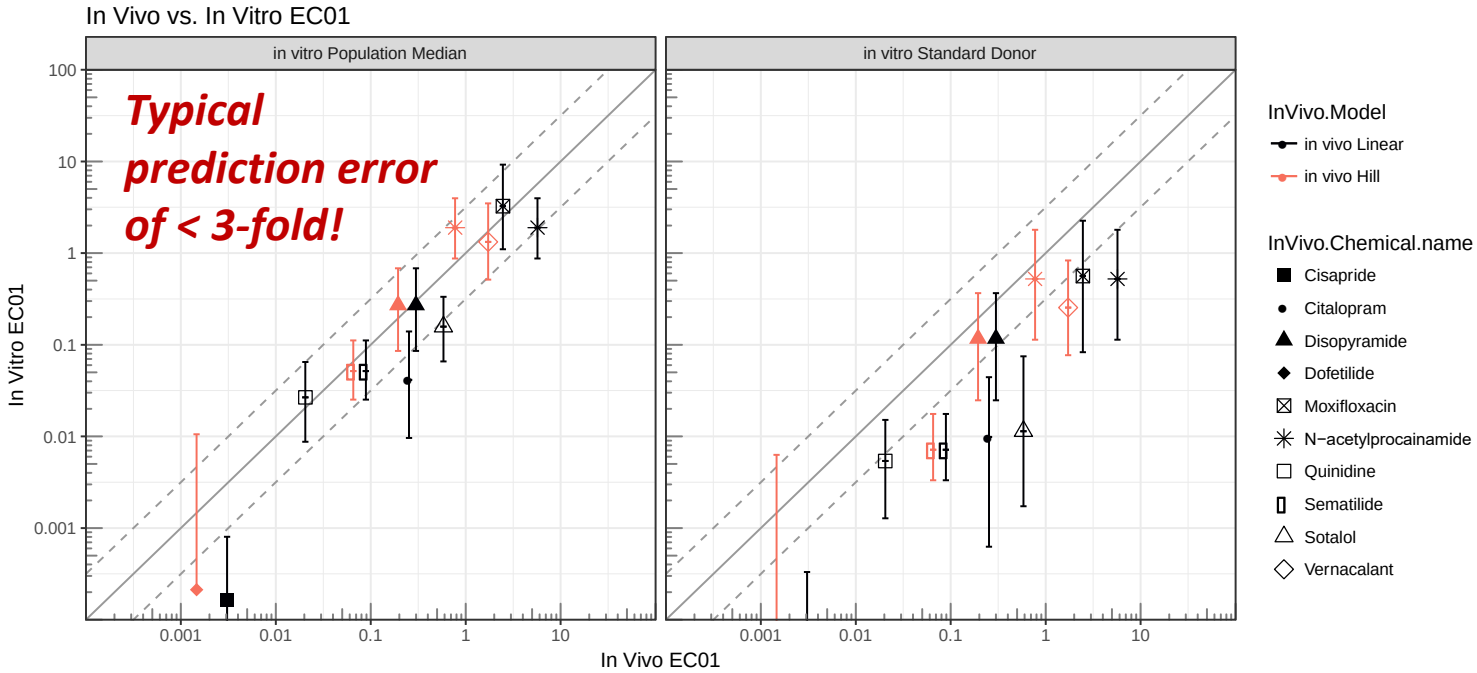

## **Negative** Control




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

Blanchette et al., 2019

# Results:
# **Quantitative** Predictions (Risk)



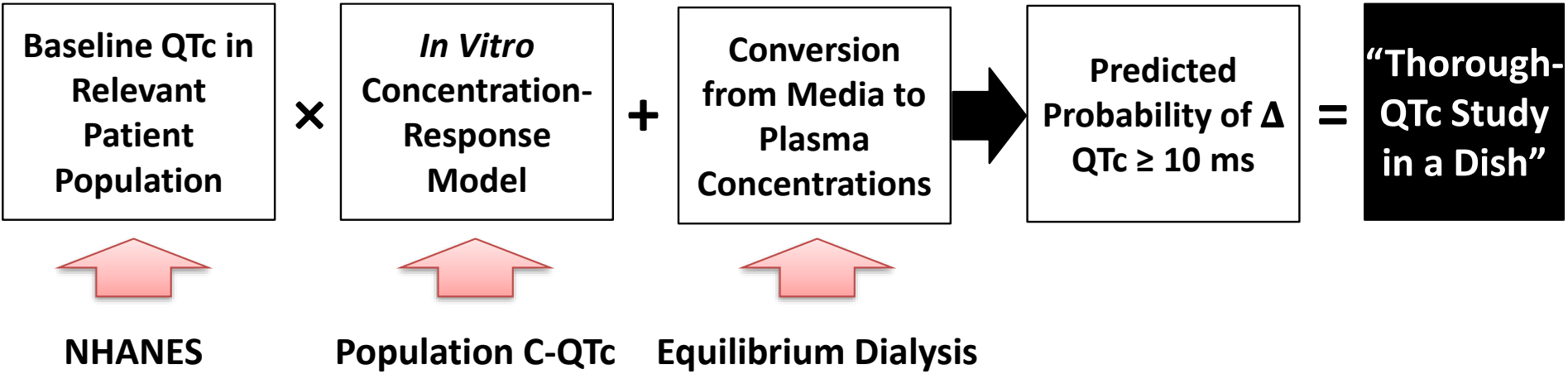

EC01=1% change

Clinical guidance:
95% for 10 ms change →
Mean change of 5 ms →
1.2% change from baseline (based on NHANES data)

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

Blanchette et al., 2019

# Clinical Translation



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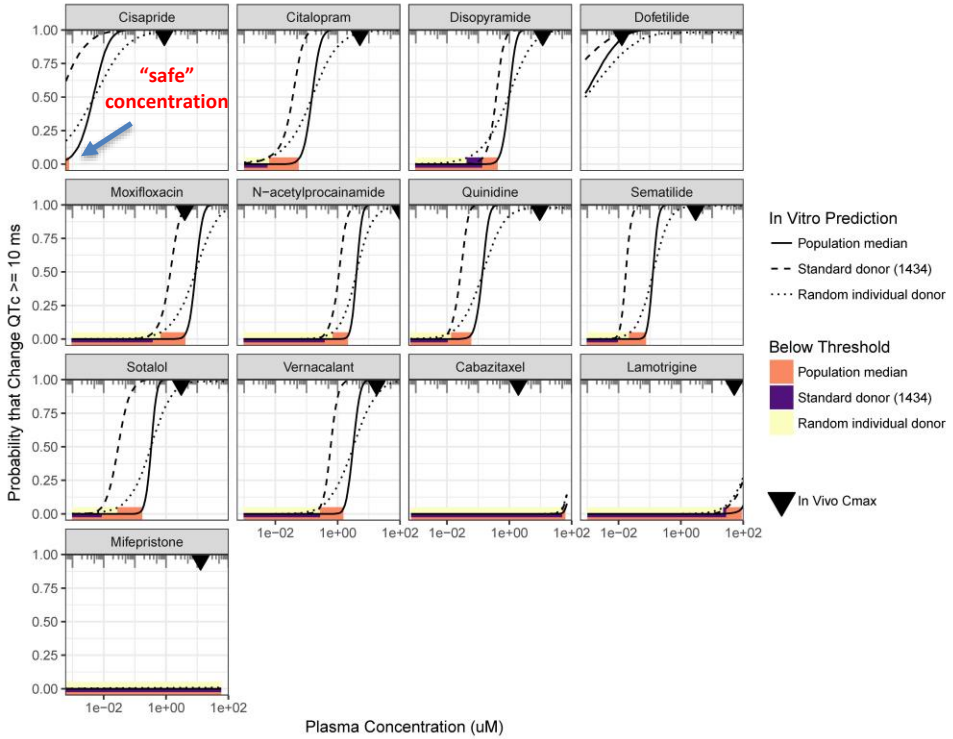
# Results: Clinical Translation




- Clinical translation of *in vitro* C-QTc modeling results involves determining the probability that clinical $\Delta QTc(x_{plasma})$ is >10 ms (95%ile)
- 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)

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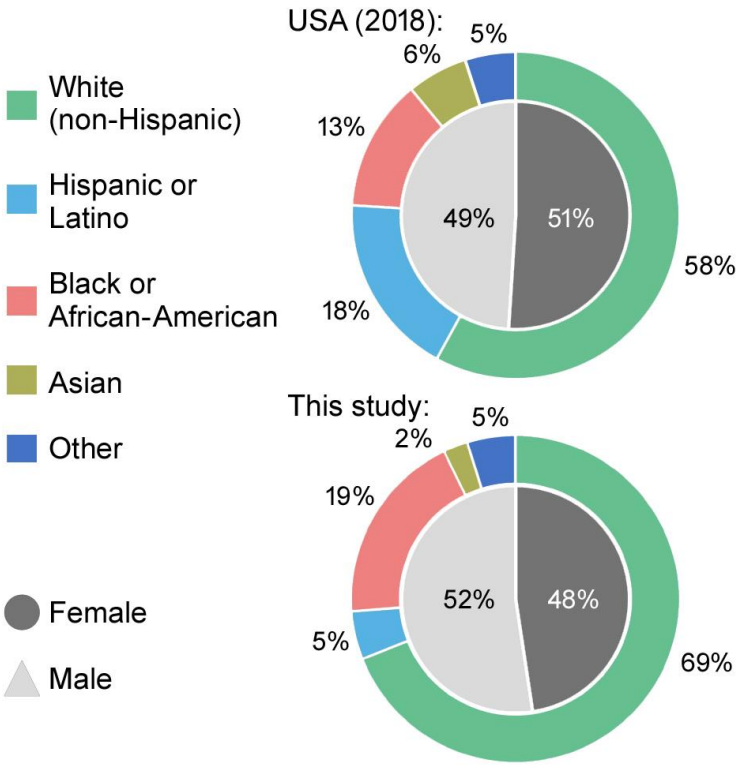
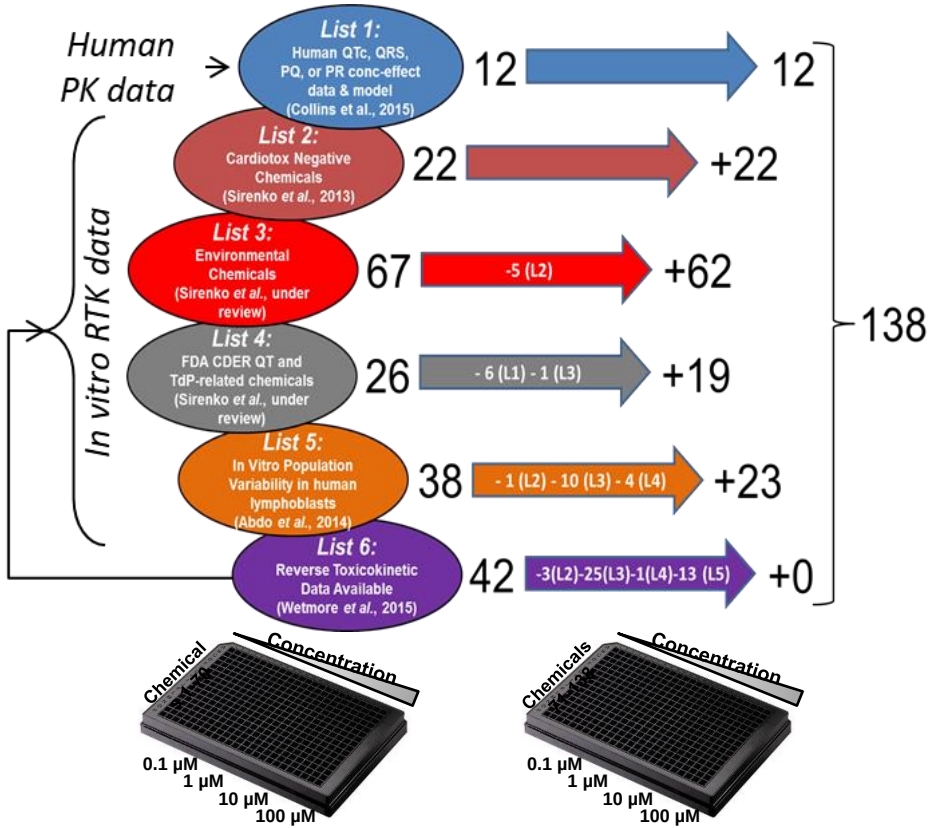
# Demonstrating the Throughput of the Population-Based Model




**N=43** Humans (all cells available from FCDI)




**N=138** Chemicals (drugs and environmental)



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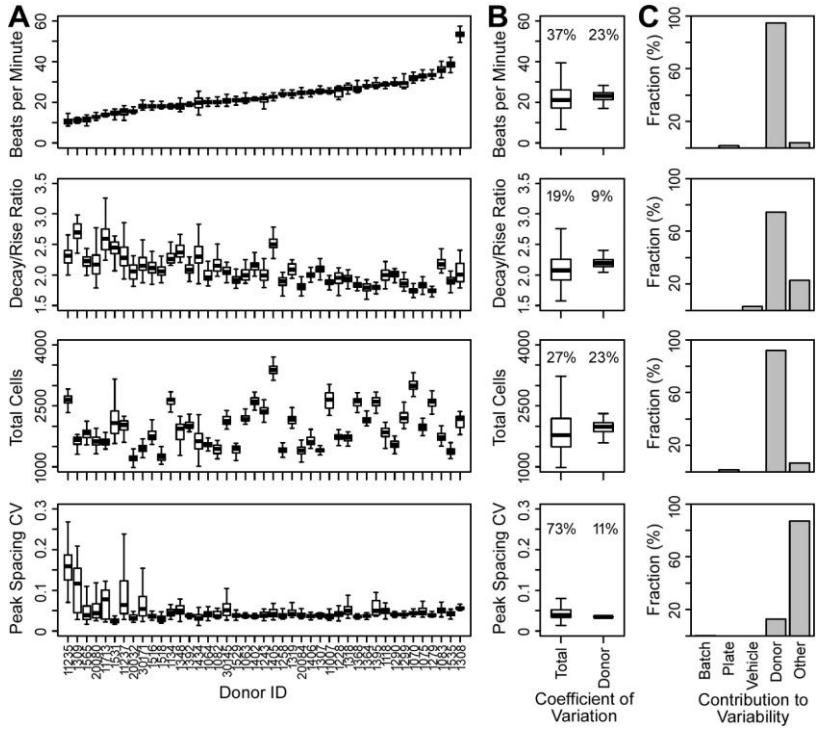
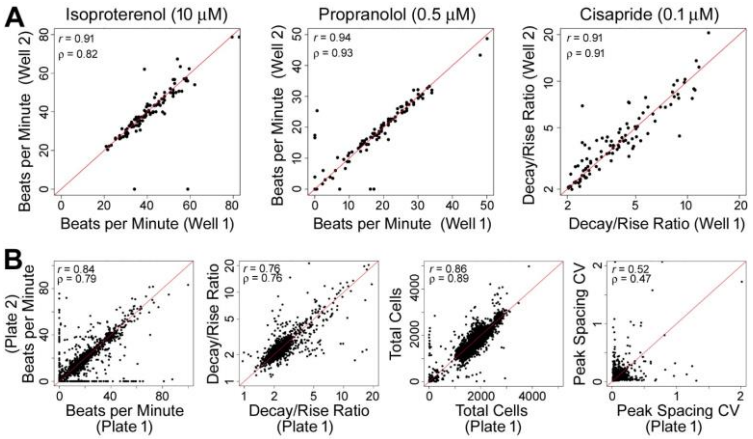
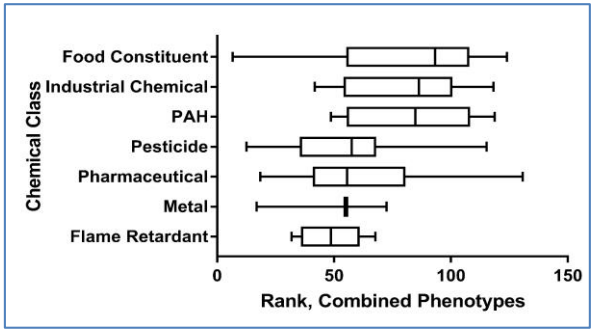
# Baseline and chemical-induced variability across cell lines is ***biological*** and ***reproducible*** *(43 donors)*



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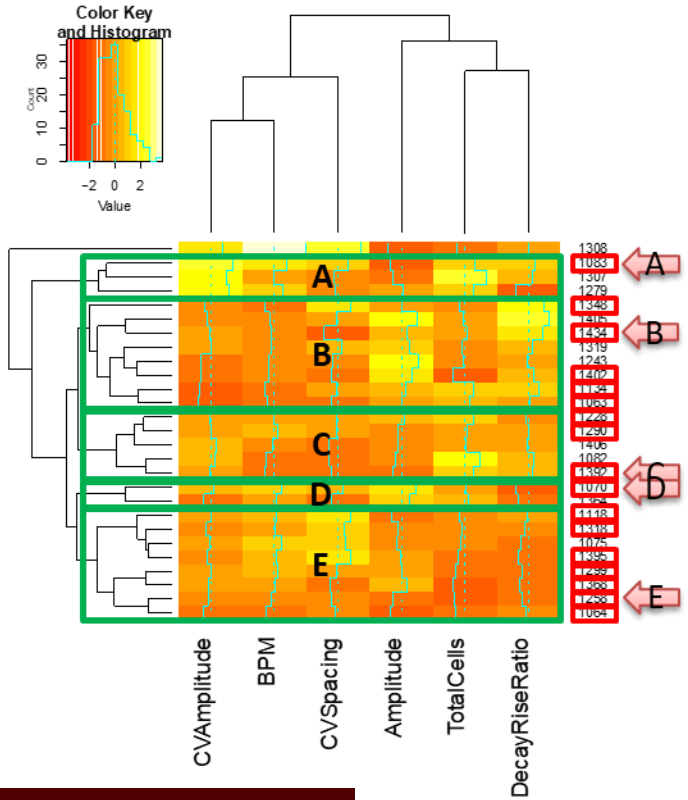
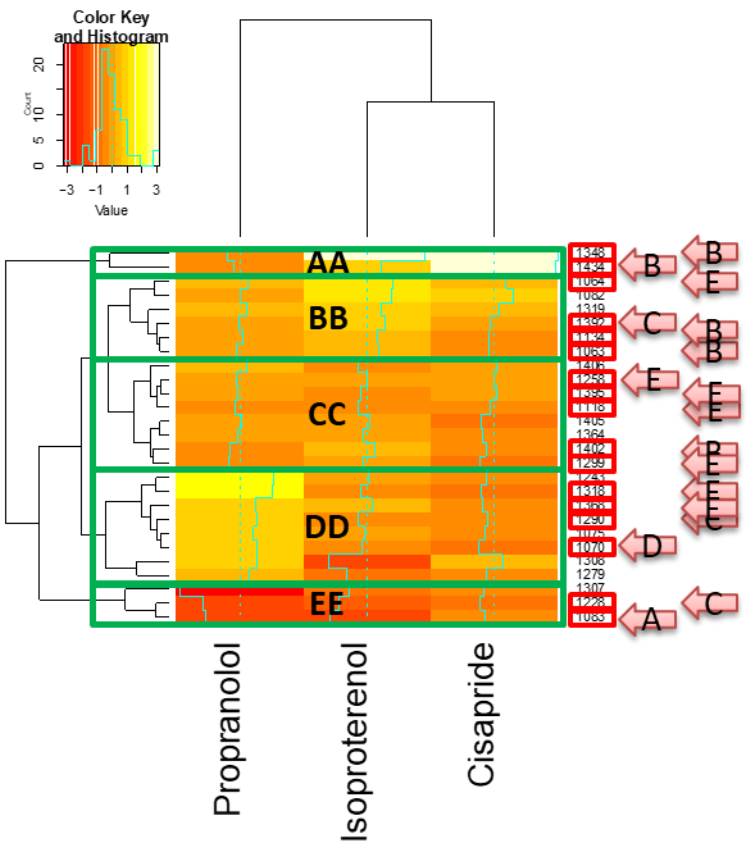
# High-Throughput of the Population-Based Model: Donors

Baseline Characteristics (Median for each Cell)




Treatment-related Characteristics (PRO, ISO, CIS)




**Selected 5 Donors:**

**1070**

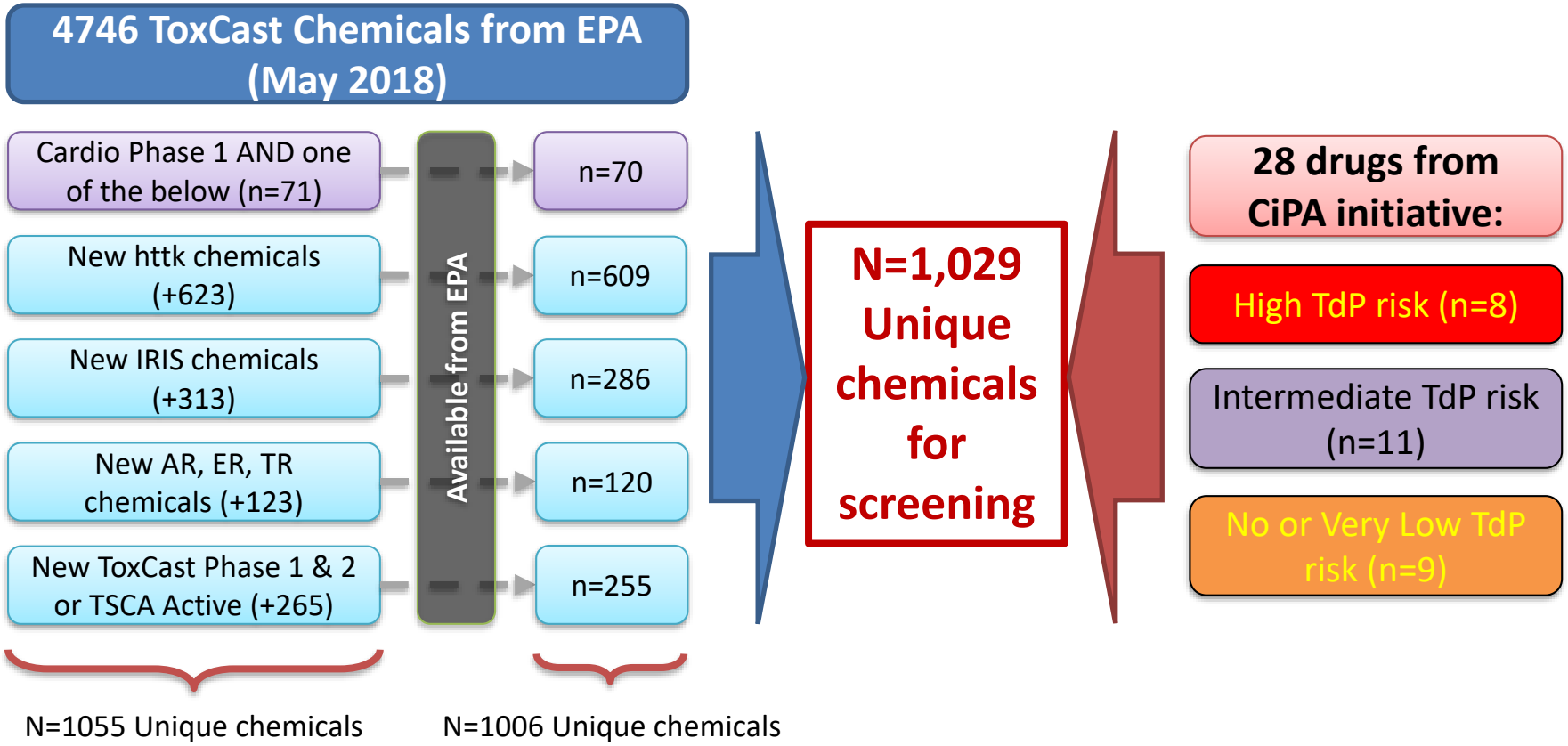
**1083**

**1258**

**1392**

**1434**

# High-Throughput of the Population-Based Model: Chemicals



# Relevance of this program to the EPA

- Established **a model** for testing potential cardiotoxicity of environmental chemicals (**none exists** now even in ToxCast)
- Showed that this human *in vitro* model is physiological, human relevant, reproducible, and **high-throughput**
- Demonstrated that this model can be used to quantify **population variability** in responses to chemicals
- Showed how this *in vitro-in silico* model can make **clinically-relevant predictions for chemical effects** on the heart rhythm

NAMs in Action