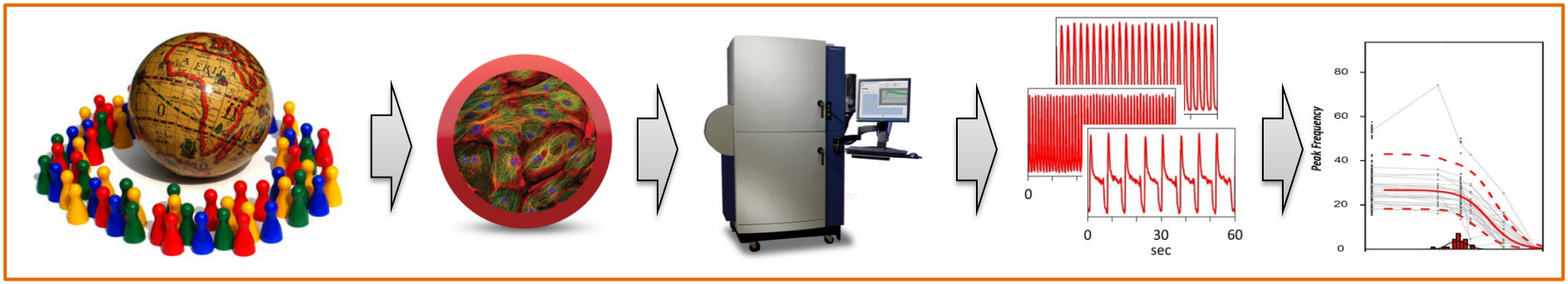
Cardiotoxicity Adverse Outcome Pathways
C-AOP STAR Center
- Project 1 Progress Report -



Diversity in a dish: A population-based organotypic human in vitro model for cardiotoxicity testing

Fabian Grimm

Department of Veterinary Integrative Biosciences
Texas A&M University

VETERINARY MEDICINE & BIOMEDICAL SCIENCES
TEXAS A&M UNIVERSITY

E-mail: fgrimm@cvm.tamu.edu

Acknowledgements

Texas A&M University

Ivan Rusyn, MD PhD
Weihsueh Chiu, PhD
David Threadgill, PhD
William Klaren, PhD
Yasuhiro Iwata, DVM
Sarah Burnett
Alec Wright

North Carolina State University

Fred Wright, PhD
David Reif, PhD
John House, PhD

Cellular Dynamics International

Blake Anson, PhD

Molecular Devices LLC

Oksana Sirenko, PhD

National Toxicology Program/ NIEHS

Raymond Tice, PhD
Kristen Ryan, PhD
Mamta Behl, PhD
Frederick Parham, PhD

Funding

U.S. Environmental Protection Agency:
STAR RD83516602 / RD83580201

Society of Toxicology:
2015 Colgate-Palmolive Fellowship
2017 Syngenta Fellowship

Introduction: Bridging Emerging Technologies And Chemical Safety Assessments

Advances in stem cell technologies and organotypic culture methods have the potential to overcome major limitaitons in contemporary risk assessment:

- Limited interpretability of animal model-derived data
- Low-throughput asssociated with *in vivo* testing ("Chemical Data Gap")
- Standardized, rather than chemical-specific population-level adjustment factors

The implementation of organotypic culture models in human health safety assessments is impeded by the lack of multidimensional high-throughput testing strategies that are:

- Functionally and physiologically-relevant[1,2] ✓
- Medium- to high-throughput applicable format[1,2] ✓
- Amenable for *in vitro*-to-*in vivo* extrapolation (physiologically-relevant exposure levels)[2] ✓
- Capable of estimating inter-individual susceptibilities to adverse chemical effects ?

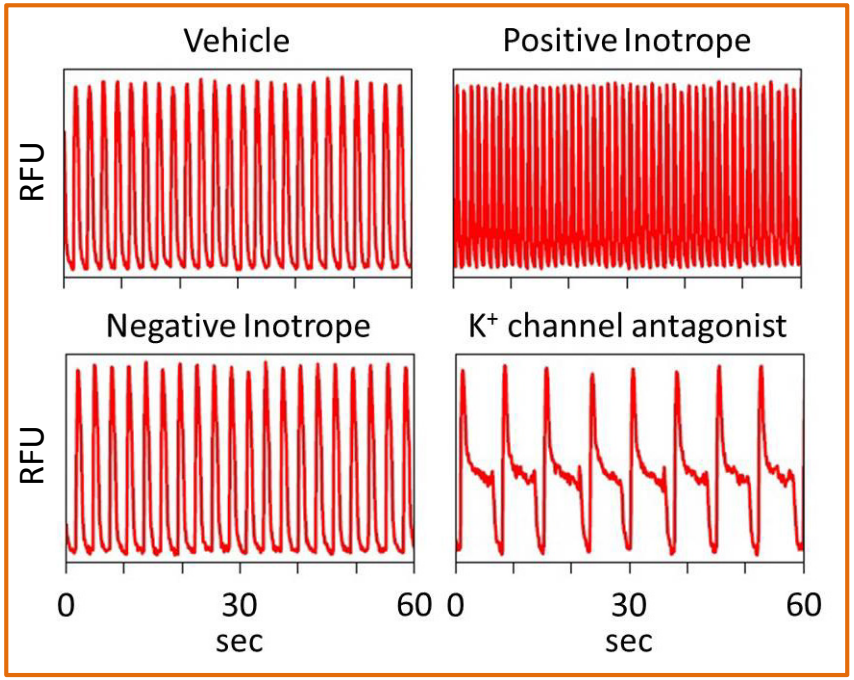
Goal: Demonstrate the potential of organotypic culture systems to fill crucial needs in chemical risk assessment using a population-based in vitro cardiotoxicity model

1. Grimm FA, Iwata Y, Sirenko O, Bittner M, Rusyn I. High-content assay multiplexing for toxicity screening in induced pluripotent stem cell-derived cardiomyocytes and hepatocytes. Assay Drug Dev Technol. (2015) 13: 529-46

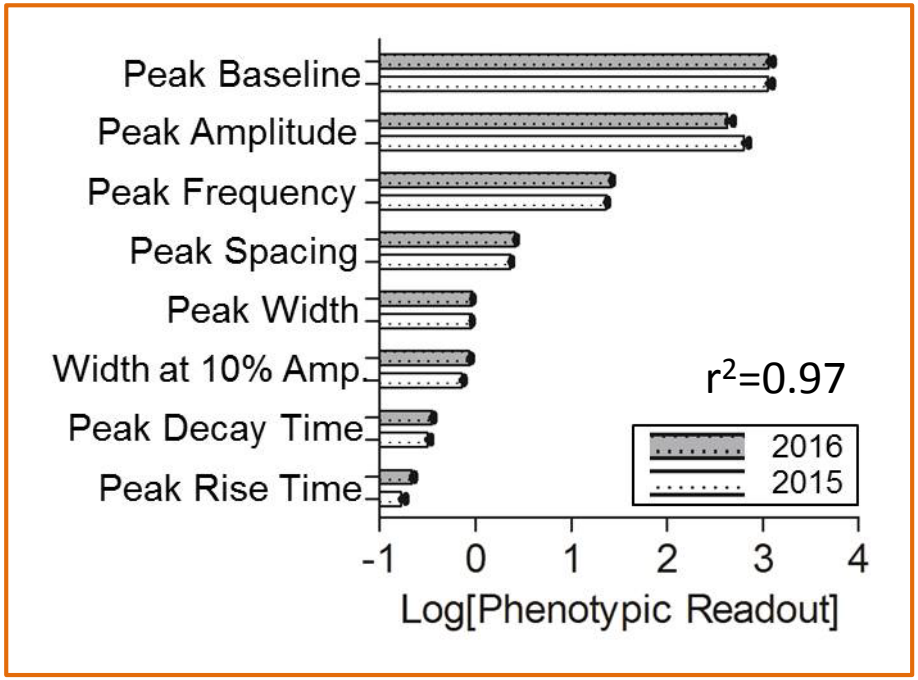
2 .Sirenko O, Grimm FA, Ryan KR, Iwata Y, Behl M, Wignall JA, Parham F, Anson B, Cromwell EF, Rusyn I, Tice RR. In vitro cardiotoxicity assessment of environmental chemicals using an organotypic human induced pluripotent stem cell-derived model. Toxicol Appl Pharm. (2017) In Press.

iPSC cardiomyocytes: A human organotypic in vitro model for cardiotoxicity testing

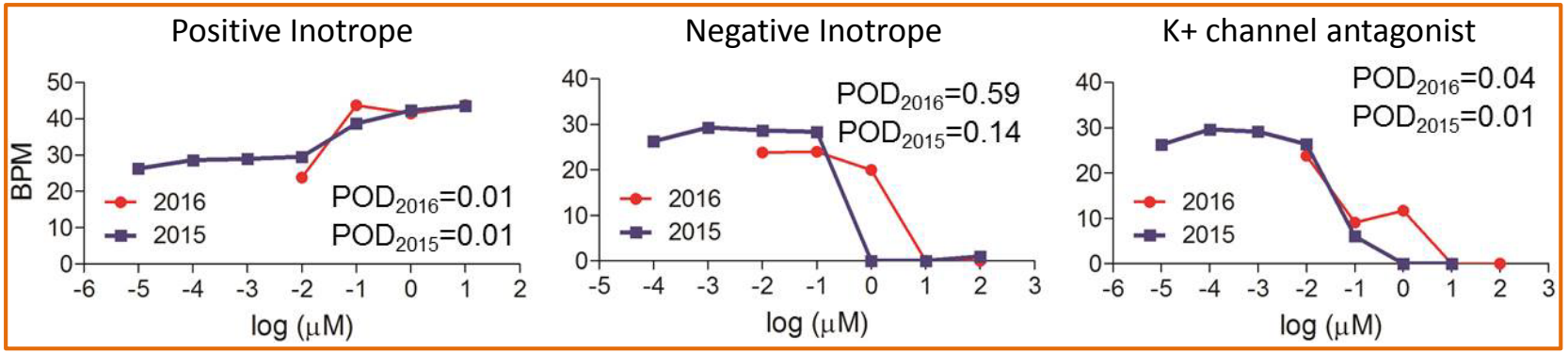
Phenotypic Resemblance of In Vivo Drug Effects



Reproducibility of Baseline Cardiophysiology



Reproducibility of Chemical Effects in iCell Cardiomyocytes

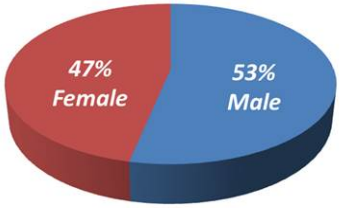


Population Variability Assessment in iPSC Cardiomyocytes: Study Design and Data Integration

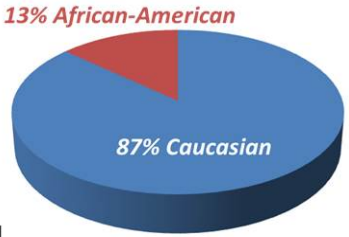
Diversity in a Dish Concept for Cardiotoxicity Testing

Donor Pool
27 individual, "healthy" donors

Gender Distribution



Origin/ Ethnicity



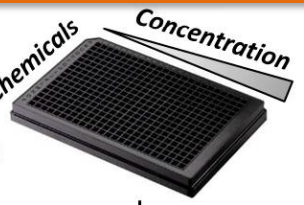
iPSC reprogramming and differentiation

Cellular Dynamics international
a FUJIFILM company

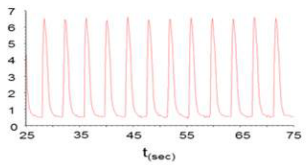
Population Variability Screening

Experimental Design

Screening Library:
>100 Pharmaceutical & Environmental Chemicals

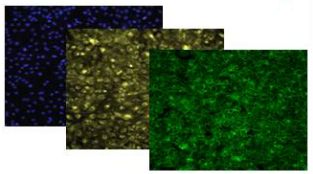


Functional Cardiophysiology



Calcium-Flux

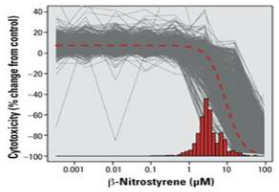
Cellular/ Mitochondrial Toxicity



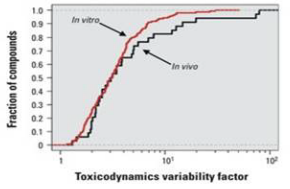
High-Content Imaging

Inherent variability in untreated CMs & Concentration-Response Assessment

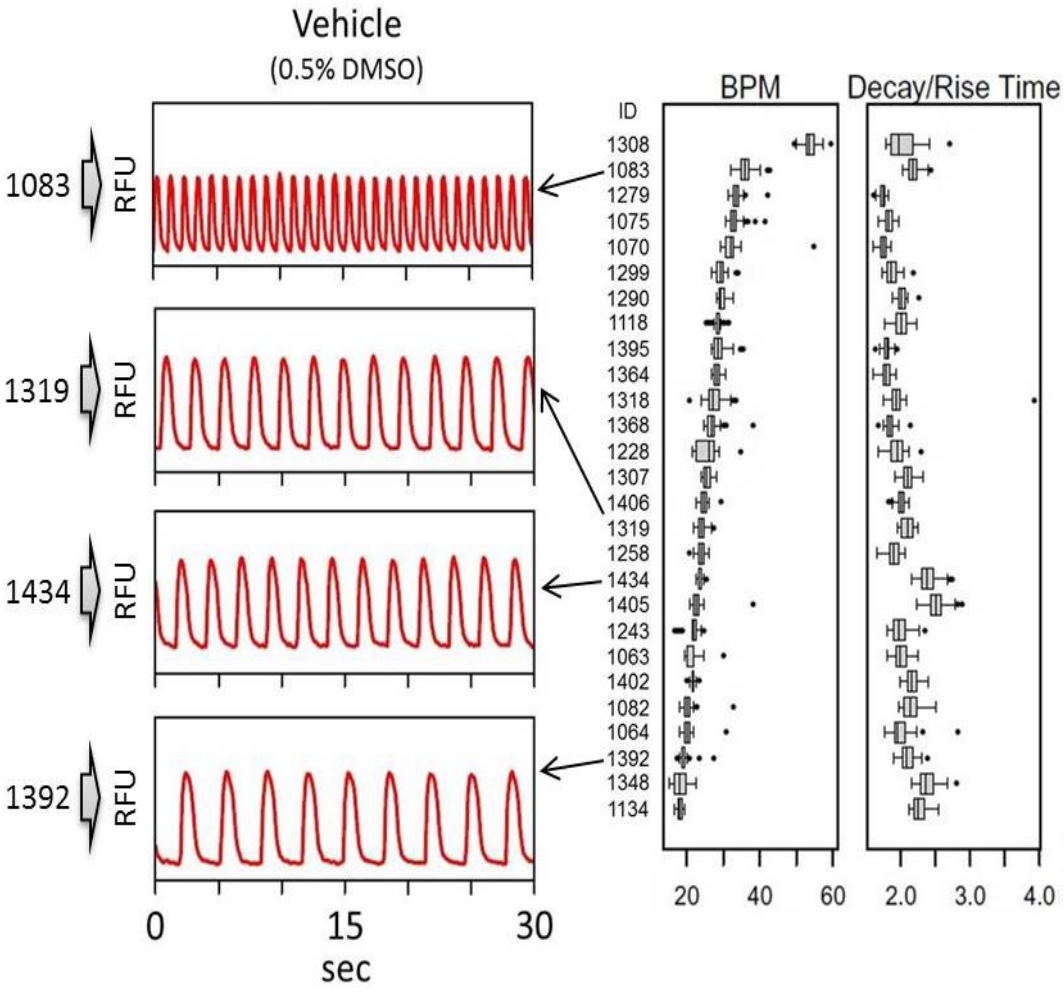
Population Variability Assessment



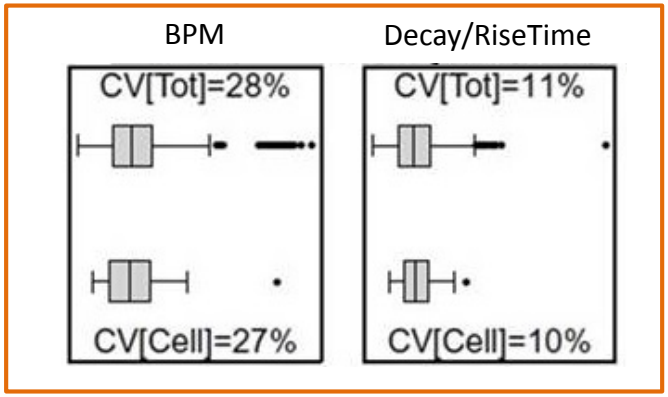
In Vitro-to-In Vivo Extrapolation



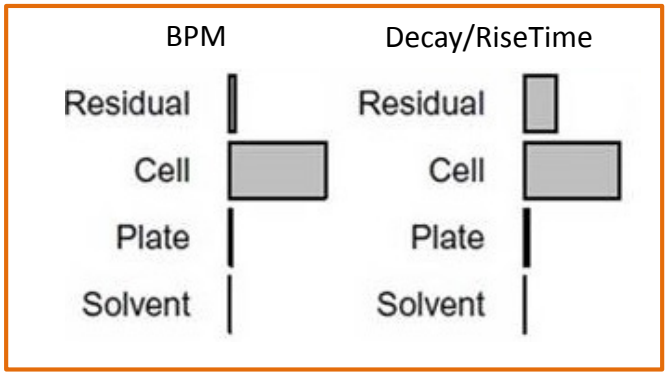
Ca^{2+} Flux is a Cardiophysiology Indicator with Individual Specificity in Untreated Cardiomyocytes



Estimated biological vs total observable variation

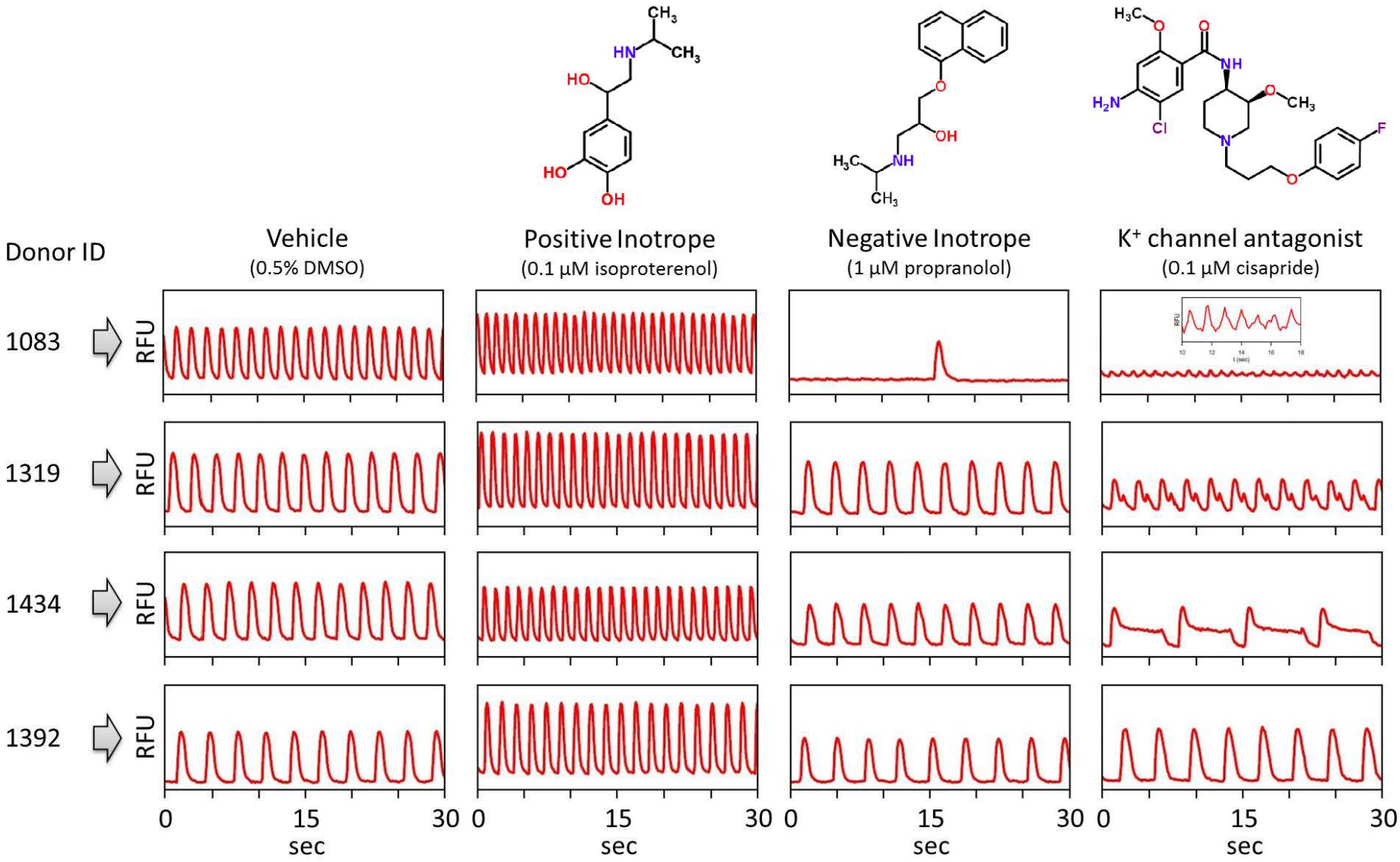


Relative contribution to observable variation



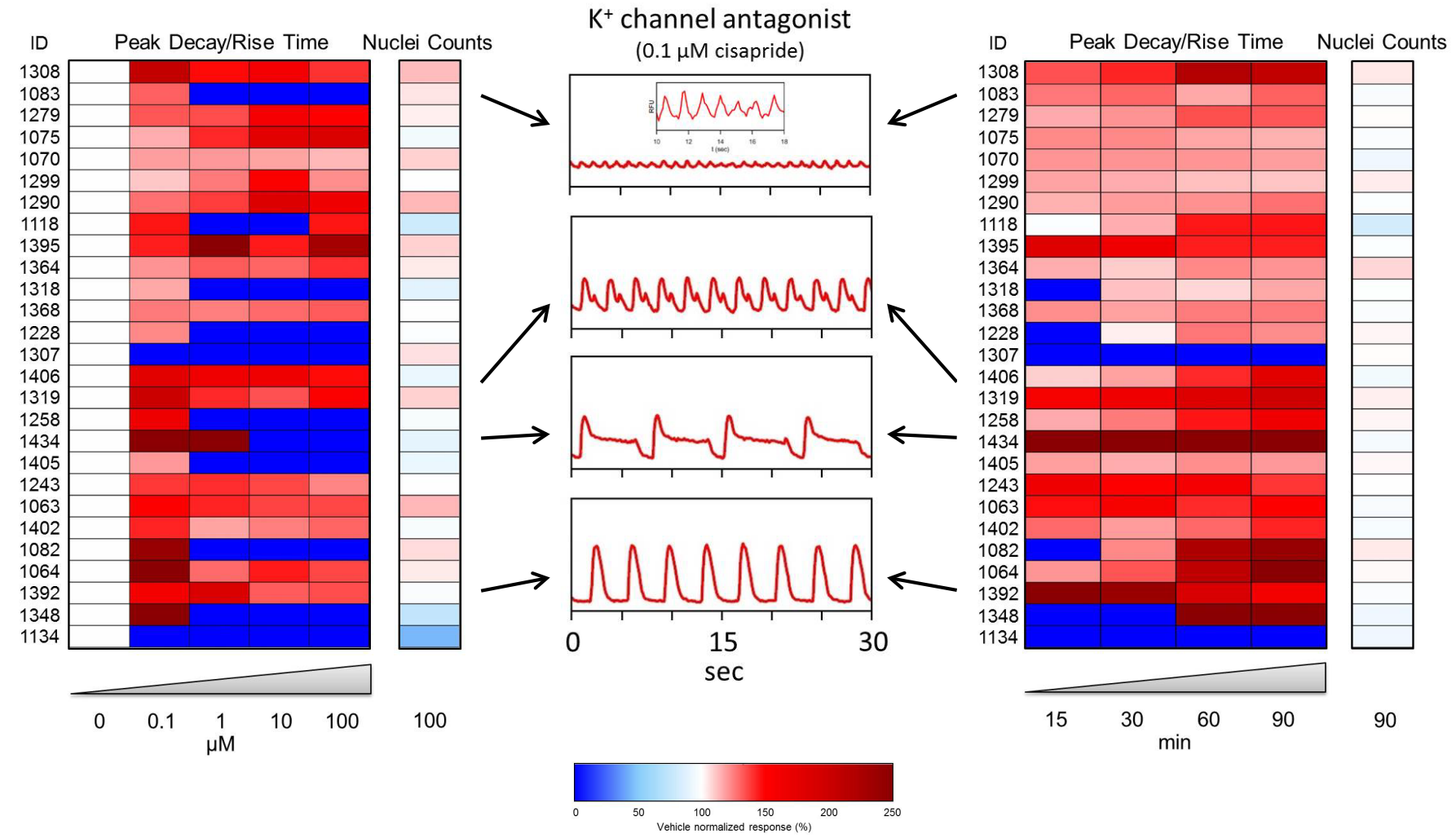
Cardiophysiologic Paramters Beat Frequency and Decay/Rise Time show ranges of inter-individual variation

Inter-Individual Differences in Baseline Cardiophysiology are Reflected in Phenotypic Responses to Chemical Treatments



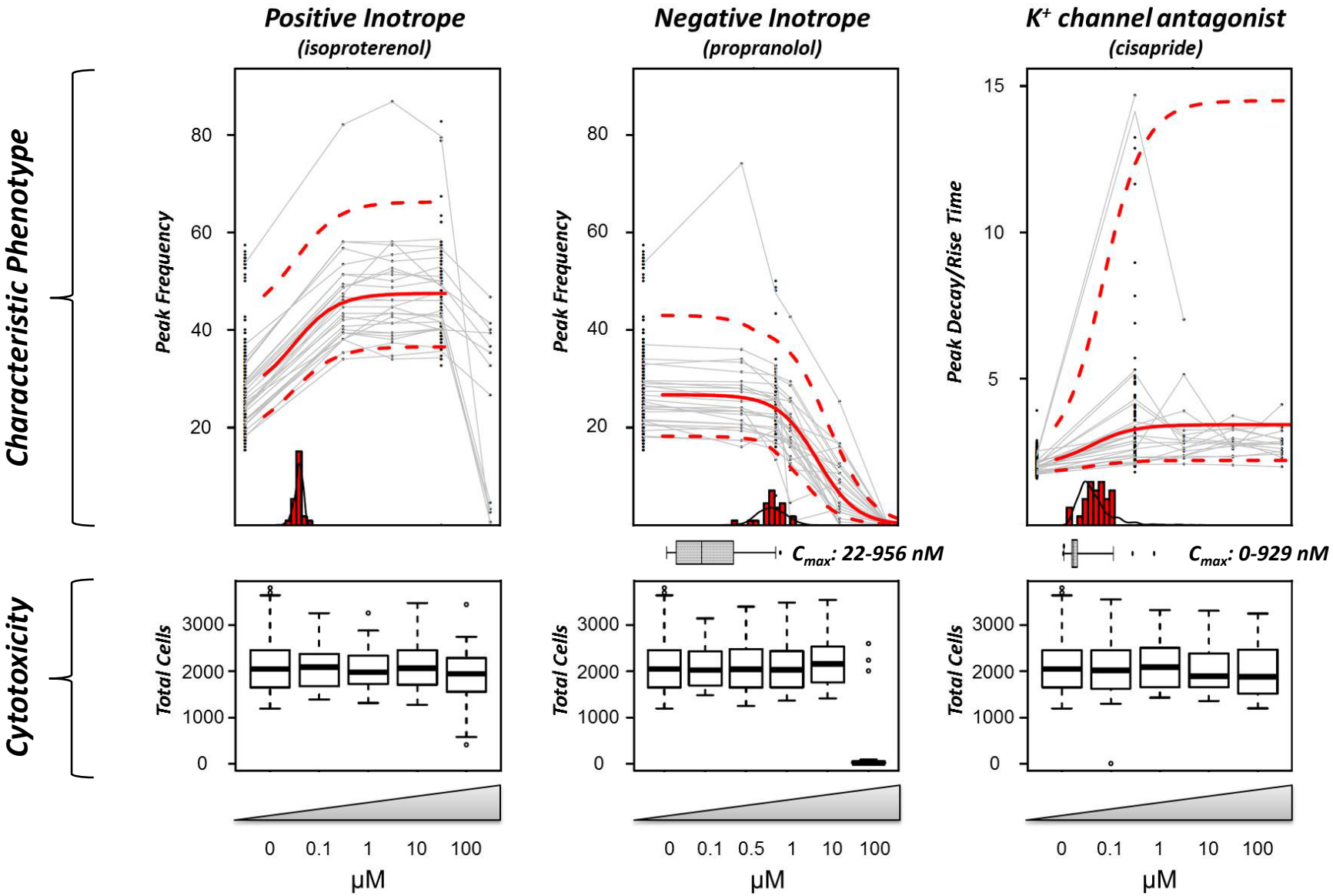
Anticipated phenotypic responses are observable upon chemical treatment. Qualitative and quantitative variation among different cardiomyocyte donors IDs is observable

Inter-Individual Differences in Baseline Cardiophysiology are reflected in concentration-responses to chemical treatments



Normalized concentation-responses for phenotypic reference chemicals show a range of inter-individual variability

Population-Level Concentraiton-Response Assessment & In Vitro-to-In Vivo Extrapolation



Population-level concentration-response data are amenable for IVIVE and derivation of chemical specific adjustment factors.

Summary & Outlook

1. Collected Ca^{2+} flux and HC-Imaging data plus cell lysates for HT-transcriptomics in concentration-response for ~140 chemicals in iPSC-derived cardiomyocytes

- *Data acquisition is complete for first batch of cells from 27 donors*
- *Identical data sets will be generated for cells from an additional 70 donors*
- *HT-transcriptomics currently underway*

2. Observable variability in baseline cardiophysiological parameters

- *not attributable to technical variation in plating efficiencies*
- *is an important factor to be considered for evaluation of chemical effects*

3. Chemical treatments qualitatively reflect the anticipated phenotypic responses

- *Qualitative characteristics remain consistent for the vast majority of chemicals*

4. Quantitative variation in responses to chemical treatments is observable

- *Differences in chemical-associated potencies are an indicator of biol. variability*