A Targeted Metabolomics-Based Assay Using Human Induced Pluripotent Stem Cell-Derived Cardiomyocytes Identifies Structural and Functional Cardiotoxicity Potential



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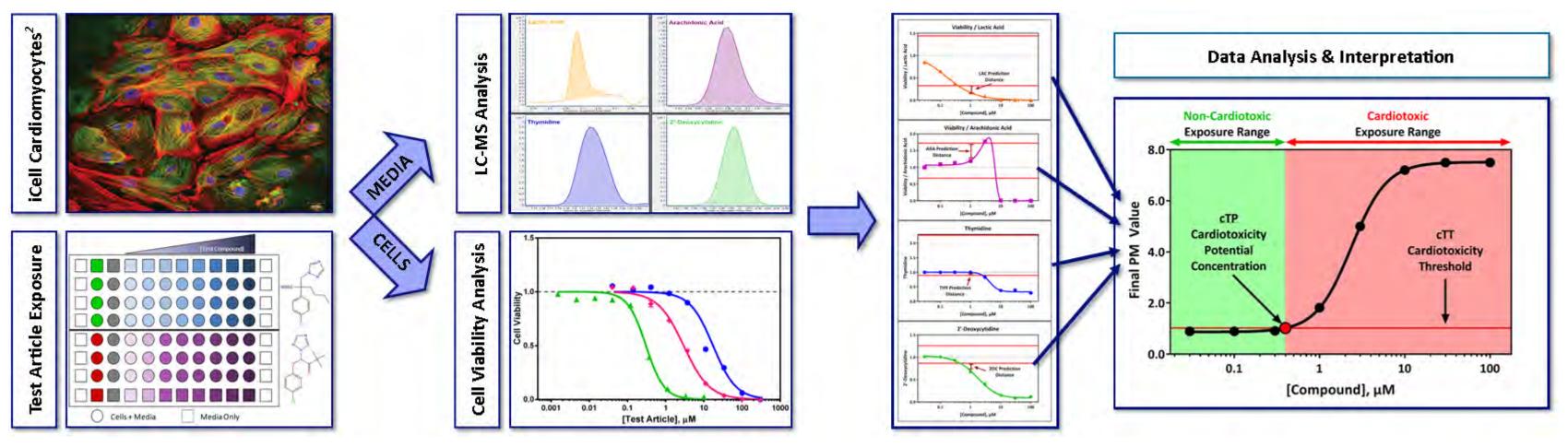
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# ABSTRACT

- Implementing screening assays that identify functional and structural cardiotoxicity earlier in the drug development pipeline has the potential to improve safety and the cost and time required to bring new drugs to market.
- In this study, a metabolic biomarker-based assay, Cardio quickPredict (Cardio<sup>qP</sup>), was developed that predicts the cardiotoxicity potential of a drug based on changes in the metabolism and viability of human induced pluripotent stem cell-derived cardiomyocytes (hiPSC-CM). Assay development and testing was conducted in two phases: (1) biomarker identification and (2) targeted assay development (Palmer et al., 2020).
- In the first phase, metabolomic data from hiPSC-CM spent media following exposure to 66 drugs was used to identify biomarkers that identified both functional and structural cardiotoxicants. Four metabolites that represent different metabolic pathways (arachidonic acid, lactic acid, 2'-deoxycytidine, and thymidine) were identified as indicators of cardiotoxicity.
- In phase two, a targeted, exposure-based biomarker assay was developed that measured these metabolites and hiPSC-CM viability across an eight-point concentration curve. Metabolite-specific predictive thresholds for identifying the cardiotoxicity potential of a drug were established and optimized for balanced accuracy or sensitivity.
- When predictive thresholds were optimized for balanced accuracy, the assay predicted the cardiotoxicity potential of 81 drugs with 86% balanced accuracy, 83% sensitivity, and 90% specificity.
  Alternatively, optimizing the thresholds for sensitivity yields a balanced accuracy of 85%, 90% sensitivity, and 79% specificity.
- This new hiPSC-CM-based assay provides a paradigm that can identify structural and functional cardiotoxic drugs that could be used in conjunction with other endpoints to provide a more comprehensive evaluation of a drug's cardiotoxicity potential.

**METHODS** 

## Cardio quickPredict Workflow



### Cardio quickPredict: Exposure-Based Prediction of Cardiotoxicity

- \* The prediction model (PM) is based on the response of four metabolites (alone or normalized to viability): lactic acid (LAC), arachidonic acid (ARA), thymidine (THY), and 2'-deoxycytidine (2DC).
- \* Metabolite-specific thresholds were previously set with diverse training set of drugs and optimized for balanced accuracy. These thresholds are used to calculate the prediction distance for each metabolite based on the difference between the threshold and the hiPSC-CM response at a given concentration.
- \* The final PM value is the maximum prediction distance at each concentration. These values are used to determine the concentration where a drug alters hiPSC-CM metabolism at a level indicative of cardiotoxicity potential.
- \* The <u>Cardiotoxicity Potential Concentration</u> (cTP) is predicted based on the point where the concentration-response curve for the final PM values crosses the <u>Cardiotoxicity Threshold</u> (cTT).
- Human iPSC-CM (iCell Cardiomyocytes<sup>2</sup>, FUJIFILM Cellular Dynamics, Inc.) were plated in 96-well plates and exposed to drug for 72 hours.
- Spent media from the last 24-hour treatment period was collected for UPLC-HRMS analysis and cell viability was assessed with the CellTiter-Fluor Cell Viability Assay (Promega).
- Samples were analyzed with rapid UPLC-HRMS method optimized for the predictive metabolites .
- Each metabolite was normalized to the reference treatment samples (0.1% DMSO). Non-linear concentration-response curves were fit for each endpoint using GraphPad Prism.
- The prediction distance for each metabolite in the Cardio quickPredict PM was calculated at each concentration and the final PM value was determined.
- The final PM values were fit with a non-linear concentration-response curve and the "Cardiotoxicity Potential Concentration" (cTP) was predicted based on the point where the concentration response curve crossed the "Cardiotoxicity Threshold".

# RESULTS

# <u>Cardio<sup>qP</sup> Accurately Predicts Functional and Structural Cardiotoxicants</u>

## Cardio<sup>9<sup>P</sup> Predictions for 81 Drugs at 10×Cmax for the Prediction Model Trained for Balanced Accuracy (BAC) and Sensitivity (SEN)</sup>

Drug	BAC-Trained: Cardio <sup>qP</sup> Model		Drug	BAC-Trained: Cardio <sup>qP</sup> Model	SEN-Trained: Cardio <sup>qP</sup> Model	Drug	BAC-Trained: Cardio <sup>qP</sup> Model	SEN-Trained: Cardio <sup>qP</sup> Model	Drug	BAC-Trained: Cardio <sup>qP</sup> Model	SEN-Trained: Cardio <sup>qP</sup> Model	Drug	BAC-Trained: Cardio <sup>qP</sup> Model	SEN-Trained: Cardio <sup>qP</sup> Model
Acetylsalicylic A	cid Non	Non	Leucine	Non	Non	Astemizole	Тох	Тох	Azidothymidine	Tox	Тох	Amiodarone	Тох	Тох

Acyclovir	Non	Non	Loratadine	Non	Non		Bepridil	Тох	Тох		Busulfan	Тох	Тох		Amitriptyline	Тох	
Adipic Acid	Non	Non	Maltol	Non	Non	s.	Chlorpromazine	Тох	Тох		Daunorubicin	Тох	Тох	S	Amphotericin B	Тох	
Amoxicillin	Non	Non	Methylparaben	Non	Non	Z	Cisapride	Тох	Тох	ITS	Dexfenfluramine	Non	Non	A	Amsacrine	Тох	
Ascorbic Acid	Тох	Тох	Natamycin	Non	Non	<u>S</u>	Dofetilide	Тох	Тох	A	Dithiazanine lodide	Тох	Тох	S	Anagrelide	Non	
Aspartame	Non	Non	Phenylphenol	Non	Non	õ	Encainide	Non	Тох	X	Doxorubicin	Тох	Тох	<u>ê</u>	Arsenic Trioxide	Тох	
Axitinib	Non	Non	Praziquantel	Non	Non	<u></u>	Levomethadyl	Toy	Тоу	15	Idarubicin	Тох	Тох	<u>o</u>	Bortezomib	Тох	
Benzoic Acid	Non	Non	Ranitidine	Non	Non	<b>B</b>	Acetate	10.2	10,2	B	Imatinib	Тох	Тох	<b>RD</b>	Chloroquine	Тох	
Biotin	Non	Non	Sildenafil	Non	Тох	5	Nifedipine	Тох	Тох	AR	Nandrolone	Non	Тох	2	Clozapine	Тох	
Cetirizine	Non	Non	Sucrose	Non	Non	٩	Ondansetron	Тох	Тох		Decanoate	NOT	107	<b>M</b>	Crizotinib	Тох	
Cimetidine	Non	Non	Tartaric Acid	Non	Non	N N	Quinidine	Тох	Тох	RA	Pergolide	Non	Non	Ξ	Dasatinib	Тох	
Citric Acid	Non	Non	Thiabendazole	Non	Тох	E	Sertindole	Тох	Тох	E	Rofecoxib	Тох	Тох		Fluorouracil	Тох	
Erlotinib	Тох	Тох	Tolbutamide	Non	Тох	Š	Sotalol	Тох	Тох	Ď	Rosiglitazone	Тох	Тох	IR	Isoproterenol	Non	
Gemfibrozil	Тох	Тох	Xylitol	Non	Non		Terfenadine	Тох	Тох	STI	Tegaserod	Non	Тох	& S	Lapatinib	Тох	
Hexylresorcinol	Non	Non				-	Thioridazine	Тох	Тох		Telmisartan	Non	Non	F	Mitoxantrone	Тох	
			-				Verapamil	Тох	Тох		Trastuzumab	Тох	Тох	Z	Nilotinib	Тох	
											Valdecoxib	Non	Non	Ĕ	Nortriptyline	Тох	
	Adipic Acid Amoxicillin Ascorbic Acid Aspartame Axitinib Benzoic Acid Biotin Cetirizine Cimetidine Citric Acid Erlotinib Gemfibrozil	Adipic AcidNonAmoxicillinNonAscorbic AcidToxAspartameNonAxitinibNonBenzoic AcidNonBiotinNonCetirizineNonCimetidineNonCitric AcidNonErlotinibToxGemfibrozilTox	Adipic AcidNonNonAmoxicillinNonNonAscorbic AcidToxToxAspartameNonNonAxitinibNonNonBenzoic AcidNonNonBiotinNonNonCetirizineNonNonCimetidineNonNonCitric AcidNonNonErlotinibToxToxGemfibrozilToxTox	Adipic AcidNonNonMaltolAmoxicillinNonNonMethylparabenAscorbic AcidToxToxNatamycinAspartameNonNonPhenylphenolAxitinibNonNonPraziquantelBenzoic AcidNonNonRanitidineBiotinNonNonSildenafilCetirizineNonNonSucroseCimetidineNonNonTartaric AcidCitric AcidNonNonThiabendazoleErlotinibToxToxToxXylitol	Adipic AcidNonNonMaltolNonAmoxicillinNonNonMethylparabenNonAscorbic AcidToxToxNatamycinNonAspartameNonNonPhenylphenolNonAxitinibNonNonPraziquantelNonBenzoic 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This study evaluated a broad range of pharmaceutical compounds, demonstrating the applicability of the Cardio<sup>*qP*</sup> assay across multiple therapeutic classes and mechanisms of cardiotoxicity. Each drug was classified as non-cardiotoxic or cardiotoxic based on the published cardiovascular effects for each drug. Cardiotoxic drugs were further stratified into three classes, functional, structural, and general (drugs that cause both structural and functional effects). The compound set contained both cardiovascular and non-cardiovascular drugs known to cause cardiotoxicity in humans, including Na<sup>+</sup>, K<sup>+</sup> and Ca<sup>2+</sup> channel blockers, antineoplastic, antiviral, cyclooxygenase-2 (COX-2) inhibitors, receptor agonists and antagonists (adreno, androgen, angiotensin II, dopamine, histamine, muscarinic, peroxisome proliferator-activated, serotonin), and tyrosine kinase inhibitors.

#### **Predictive Metabolites are Complimentary**

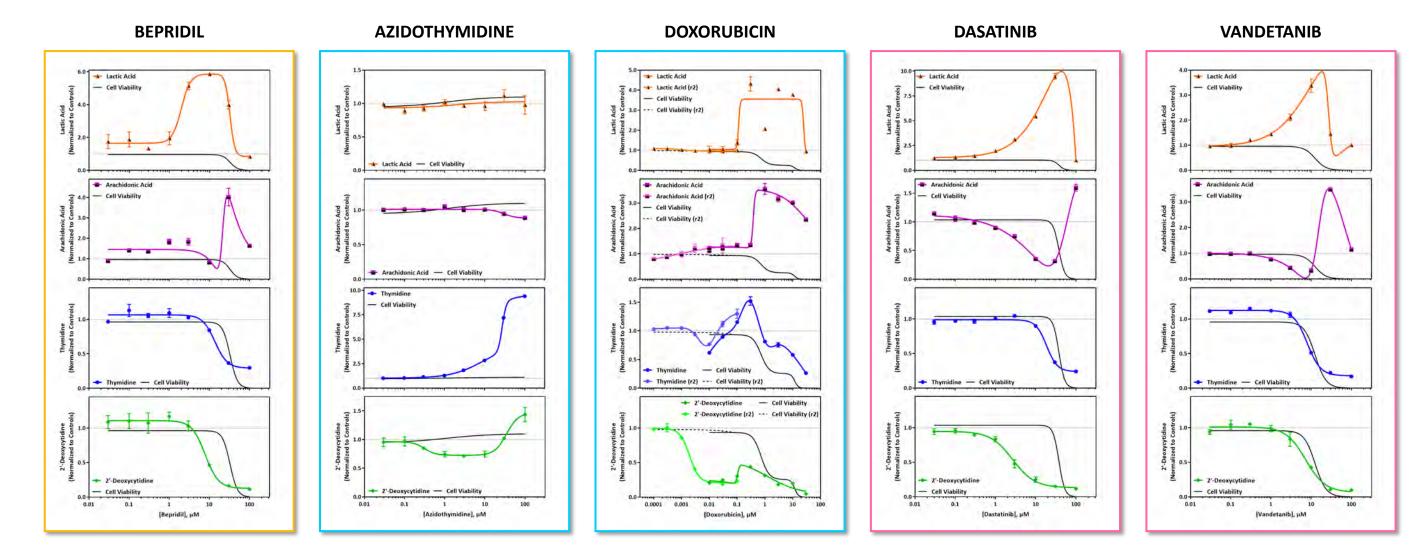
## Metabolite Concentration-Response Curves Vary between Types and Mechanisms of Cardiotoxicity

Paclitaxel

Sorafenib

Sunitinib

Vandetanib



#### **Balanced Accuracy-Trained Model Performance**

Metabolite	Balanced Accuracy	Sensitivity	Specificity	PPV	NPV	Functional*	Structural*	Functional & Structural*	
Lactic Acid	83%	65%	100%	100%	62%	80%	50%	67%	
Arachidonic Acid	74%	56%	93%	94%	54%	47%	50%	67%	
Thymidine	74%	52%	97%	96%	53%	33%	50%	67%	
2'-Deoxycytidine	81%	65%	97%	97%	61%	67%	50%	76%	
Composite Model	86%	83%	90%	93%	74%	93%	63%	90%	

#### Sensitivity-Trained Model Performance

Metabolite	Balanced Accuracy	Sensitivity	Specificity	PPV	NPV	Functional*	Structural*	Functional & Structural*	
Lactic Acid	82%	75%	90%	93%	67%	87%	56%	81%	
Arachidonic Acid	78%	69%	86%	90%	61%	53%	69%	81%	
Thymidine	76%	62%	90%	91%	57%	40%	50%	86%	
2'-Deoxycytidine	83%	73%	93%	95%	66%	87%	56%	76%	
Composite Model	85%	90%	79%	89%	82%	100%	75%	95%	

\*Percent of Subclass Correctly Predicted at Cardiotoxic

### The Metabolite Biomarkers Identified in this Study are Biologically Relevant for Cardiotoxicity

- The metabolites have key and varying roles in modulating oxidative stress and mitochondrial function and replication and are related to known mechanisms of cardiotoxicity.
- A broad range of response types was observed for each biomarker, indicating that cardiotoxic drugs may affect different components in the pathways.
- Each metabolite responds to cardiotoxicant exposure independent of changes in cell viability.

# CONCLUSIONS

- The combination of 4 metabolites predicted the cardiotoxicity potential of 81 compounds with a broad range of mechanisms and therapeutic indications with ≥85% accuracy at therapeutically relevant concentrations.
- > The Cardio<sup>*qP*</sup> assay can be used to identify both functional and structural cardiotoxicants.
- > Each metabolite can identify multiple mechanisms of cardiotoxicity.
- > The prediction model can be used to determine the concentration a compound shows the potential to cause cardiotoxicity.
- > This method can be combined with other assays or endpoints (e.g., MEA or impedance) for a comprehensive understanding of a compound's cardiotoxicity liability.
- > Future Work: Participating in the HESI multi-site study to identify chronic treatment induced cardiotoxicity using in vitro assays

Reference: Palmer, JA, et al. Toxicol Sci. 2020; 174 (2):218-240