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

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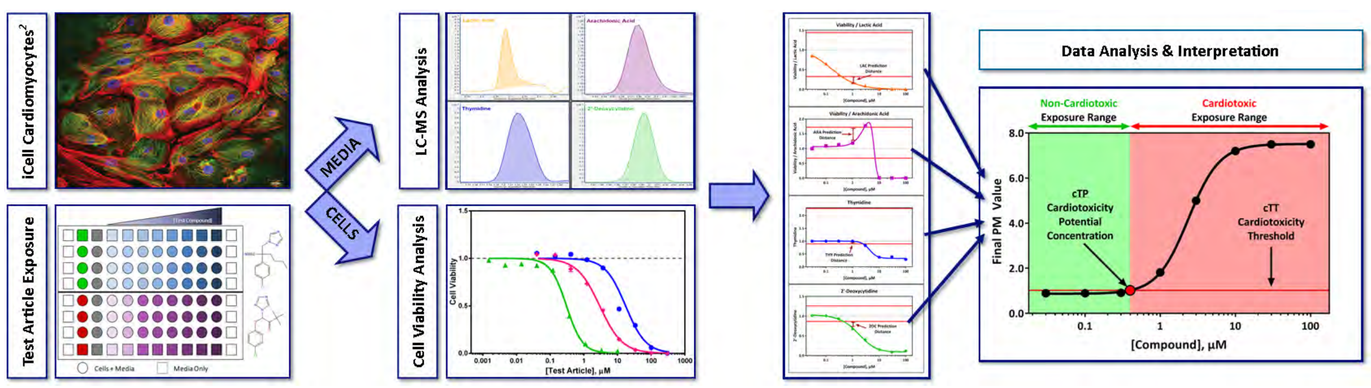
ABSTRACT

- 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 *quick*Predict (Cardio^{qP}), 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 *quick*Predict: 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 Cardiotoxicity Potential Concentration (cTP) is predicted based on the point where the concentration-response curve for the final PM values crosses the Cardiotoxicity Threshold (cTT).

- Human iPSC-CM (iCell Cardiomyocytes[2], 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 *quick*Predict 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

Cardio^{qP} Accurately Predicts Functional and Structural Cardiotoxicants

Cardio^{qP} Predictions for 81 Drugs at 10×Cmax for the Prediction Model Trained for Balanced Accuracy (BAC) and Sensitivity (SEN)

	Drug	BAC-Trained: Cardio^{qP} Model	SEN-Trained: Cardio^{qP} Model	Drug	BAC-Trained: Cardio^{qP} Model	SEN-Trained: Cardio^{qP} Model
NON-CARDIOTOXICANTS	Acetylsalicylic Acid	Non	Non	Leucine	Non	Non
	Acyclovir	Non	Non	Loratadine	Non	Non
	Adipic Acid	Non	Non	Maltol	Non	Non
	Amoxicillin	Non	Non	Methylparaben	Non	Non
	Ascorbic Acid	Tox	Tox	Natamycin	Non	Non
	Aspartame	Non	Non	Phenylphenol	Non	Non
	Axitinib	Non	Non	Praziquantel	Non	Non
	Benzoic Acid	Non	Non	Ranitidine	Non	Non
	Biotin	Non	Non	Sildenafil	Non	Tox
	Cetirizine	Non	Non	Sucrose	Non	Non
	Cimetidine	Non	Non	Tartaric Acid	Non	Non
	Citric Acid	Non	Non	Thiabendazole	Non	Tox
	Erlotinib	Tox	Tox	Tolbutamide	Non	Tox
	Gemfibrozil	Tox	Tox	Xylitol	Non	Non
	Hexylresorcinol	Non	Non			

	Drug	BAC-Trained: Cardio^{qP} Model	SEN-Trained: Cardio^{qP} Model
FUNCTIONAL CARDIOTOXICANTS	Astemizole	Tox	Tox
	Bepridil	Tox	Tox
	Chlorpromazine	Tox	Tox
	Cisapride	Tox	Tox
	Dofetilide	Tox	Tox
	Encainide	Non	Tox
	Levomethadyl Acetate	Tox	Tox
	Nifedipine	Tox	Tox
	Ondansetron	Tox	Tox
	Quinidine	Tox	Tox
	Sertindole	Tox	Tox
	Sotalol	Tox	Tox
	Terfenadine	Tox	Tox
	Thioridazine	Tox	Tox
	Verapamil	Tox	Tox

	Drug	BAC-Trained: Cardio^{qP} Model	SEN-Trained: Cardio^{qP} Model
STRUCTURAL CARDIOTOXICANTS	Azidothymidine	Tox	Tox
	Busulfan	Tox	Tox
	Daunorubicin	Tox	Tox
	Dexfenfluramine	Non	Non
	Dithiazanine Iodide	Tox	Tox
	Doxorubicin	Tox	Tox
	Idarubicin	Tox	Tox
	Imatinib	Tox	Tox
	Nandrolone Decanoate	Non	Tox
	Pergolide	Non	Non
	Rofecoxib	Tox	Tox
	Rosiglitazone	Tox	Tox
	Tegaserod	Non	Tox
	Telmisartan	Non	Non
	Trastuzumab	Tox	Tox
	Valdecoxib	Non	Non

	Drug	BAC-Trained: Cardio^{qP} Model	SEN-Trained: Cardio^{qP} Model
FUNCTIONAL & STRUCTURAL CARDIOTOXICANTS	Amiodarone	Tox	Tox
	Amitriptyline	Tox	Tox
	Amphotericin B	Tox	Tox
	Amsacrine	Tox	Tox
	Anagrelide	Non	Tox
	Arsenic Trioxide	Tox	Tox
	Bortezomib	Tox	Tox
	Chloroquine	Tox	Tox
	Clozapine	Tox	Tox
	Crizotinib	Tox	Tox
	Dasatinib	Tox	Tox
	Fluorouracil	Tox	Tox
	Isoproterenol	Non	Non
	Lapatinib	Tox	Tox
	Mitoxantrone	Tox	Tox
	Nilotinib	Tox	Tox
	Nortriptyline	Tox	Tox
	Paclitaxel	Tox	Tox
	Sorafenib	Tox	Tox
	Sunitinib	Tox	Tox
	Vandetanib	Tox	Tox

This study evaluated a broad range of pharmaceutical compounds , demonstrating the applicability of the Cardio^{qP} 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^+, K^+ and Ca^{2+} 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

Balanced Accuracy-Trained Model Performance

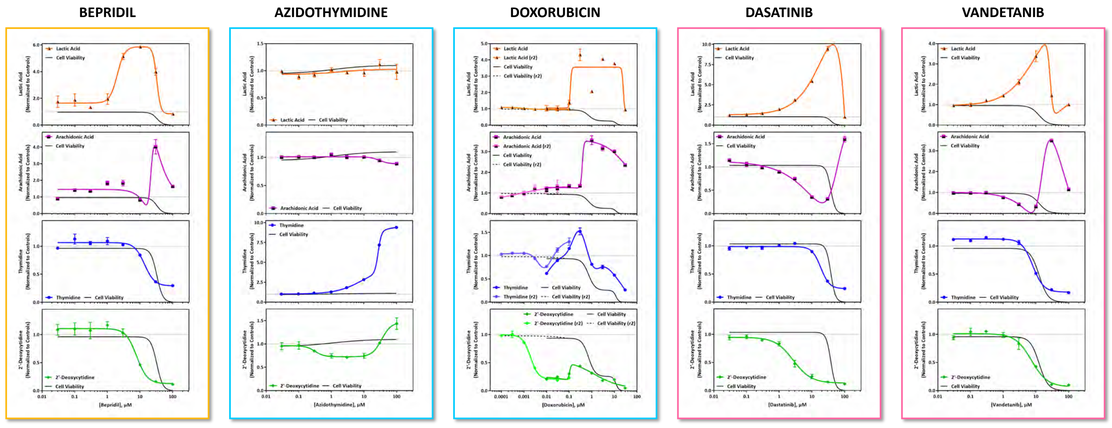
Metabolite	Balanced Accuracy	Sensitivity	Specificity	PPV	NPV	Functional*	Structural*	Functional & Structural*
Lactic Acid	83%	65%	100%	100%	62%	80%	50%	67%
Arachidonic Acid	74%	56%	93%	94%	54%	47%	50%	67%
Thymidine	74%	52%	97%	96%	53%	33%	50%	67%
2'-Deoxycytidine	81%	65%	97%	97%	61%	67%	50%	76%
Composite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

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



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^{qP} 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