Cellular Impedance assays for deconvoluting kinase inhibitor induced cardiotoxicity

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Abstract

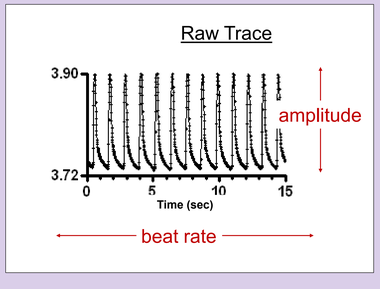
Cardiovascular (CV) toxicity is a leading contributor to drug attrition and market withdrawal. There is urgent need for earlier and broader CV screening, particularly for kinase inhibitors (KI) which suffer frequent CV toxicity. Lack of kinase selectivity is a persistent hurdle to unraveling the specific kinases that contribute to CV toxicity due to the highly conserved nature of the ATP pocket. To handle KI promiscuity and discover underling kinases requires an *in vitro* assay with the throughput to screen kinome-spanning compound sets. Addressing CV effects requires an integrated functional assay that is downstream of the excitation-contraction cascade and capable of capturing the pleiotropic effects of kinases. The emergence of cellular impedance technology enables detection of spontaneous beating of cultured cardiomyocytes (CM) in a real-time, label-free 96-well format, and can quantify both the rate and amplitude of beating. Using this technology, we are seeking to identify specific kinase profiles that underlie functional effects on CMs. The effects on iPSC-derived CM beating were evaluated using two panels of KIs (65 and 160 compounds) whose kinome selectivity has been published. Neither KI promiscuity nor physical-chemical properties appeared to correlate with changes in CM beating. The impedance and polypharmacology results are being interrogated using machine learning methods to identify culprit kinase profiles. Initial results indicate that KI promiscuity within specific kinase families (AGC, STE, and CAMK) is associated with altered CM beating. Individual kinases that have been identified as possible contributors to functional CV toxicity by recursive partitioning or feature importance algorithms are being substantiated with siRNA-based knockdown experiments. Ultimately, this study will lead to the development of a model to predict KI CV toxicity thus enabling drug discovery programs to design-in CV safety.

Background

- Cardiotoxicity is a leading cause for late stage drug attrition and withdrawal
- Several kinase inhibitors (KI) are associated with serious adverse cardiac events
- Kinases have pleiotropic cellular effects- requires assays with integrated downstream endpoints to detect perturbations in their signaling pathways
- Current *in vitro* assays focus on arrhythmia/hERG, leaving major gaps in the evaluation of other adverse effects on contractility, beat rate and toxicity

iPSC-derived Cardiomyocyte (iCM) Impedance-based Assay

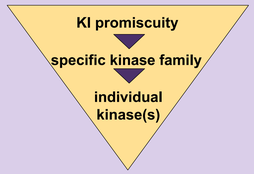
- Impedance measurements enable monitoring of CM beating in a real-time, label-free format
- Confluent monolayer of iPSC-derived CM beat spontaneously & synchronously
- Interdigitated electrodes imbedding in wells & weak alternating current impeded by cell layer
- Cell morphology & adhesion alter current flow



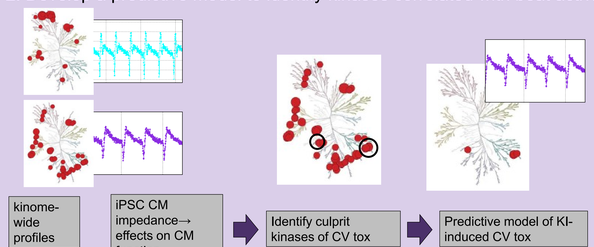
iCM impedance assay to test KI correlations and predict CV toxicity

Objectives:

1. Identify characteristics of KI that contribute to beat activity



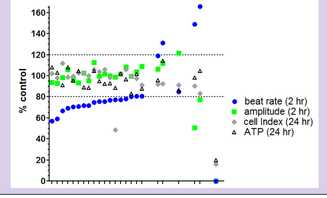
2. Develop a predictive model to identify kinases correlated with beat activity



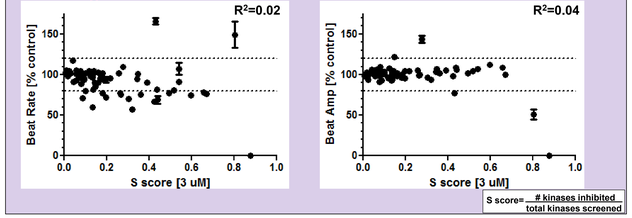
Experimental Approach

- Panel of 65 KIs whose kinome selectivity for ~385 non-redundant kinases has been published[1]
- Effects on iPSC-derived CM beating were evaluated using cellular impedance

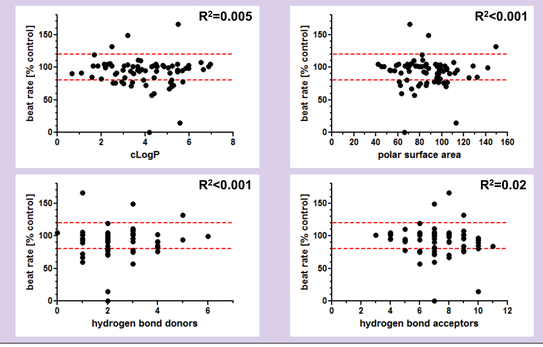
KI–induced changes of iCM beat rate and amplitude at concentrations not associated with cytotoxicity



KI selectivity (S) score is not sufficient to predict iCM beating effects



Physical-chemical properties are not sufficient to predict iCM beating effects



Promiscuity within a single kinase group not sufficient to predict iCM beating effects

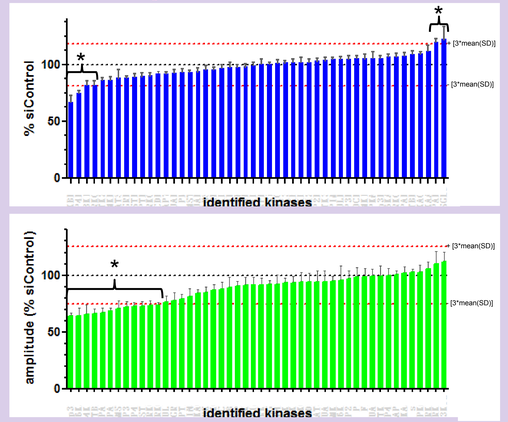
Kinase group	Enrichment score	Matthews correlation coefficient	
CAMK	2.980	0.584	Promiscuity within these groups correlates with beating effects
OTHER	2.421	0.435	
STE	2.256	0.458	
AGC	2.232	0.494	
Atypic	1.619	0.343	Promiscuity within these groups does not correlate with beating effects
CK1	1.361	0.247	
CMGC	1.349	0.255	
LIPID	1.000	0.180	
TKL	0.409	0.096	
TK	0.177	0.035	

Identification of candidate kinases correlated with iCM beating effects using feature importance algorithms

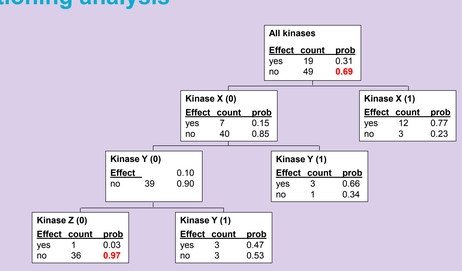
	kinase	kinase group	Enrichment score	Matthew Correlation	p-value
expressed in iCM and heart		STE	3.06	0.50	1.0E-04
		AGC	2.84	0.56	0.0E+00
		STE	2.82	0.49	1.6E-04
		AGC	2.63	0.49	2.4E-04
		TK	2.63	0.49	8.0E-05
		STE	2.55	0.42	1.2E-03
		STE	2.55	0.42	1.0E-03
		CAMK	2.54	0.56	0.0E+00
		STE	2.50	0.43	6.4E-04
		AGC	2.47	0.49	8.0E-05
		TK	2.47	0.49	1.6E-04
		TKL	2.47	0.49	1.0E-04
		OTHER	2.47	0.49	1.8E-04
		STE	2.44	0.44	4.6E-04
		STE	2.39	0.46	3.8E-04
		LIPID	2.36	0.41	1.6E-03
		CAMK	2.36	0.41	1.3E-03
		CAMK	2.36	0.41	1.3E-03
		AGC	2.36	0.41	1.5E-03
		STE	2.36	0.41	1.3E-03
		AGC	2.36	0.59	0.0E+00
		CAMK	2.34	0.49	1.0E-04
		OTHER	2.34	0.49	1.2E-04
		OTHER	2.34	0.49	1.0E-04
		STE	2.32	0.45	2.4E-04
		CMGC	2.27	0.47	2.0E-04
		AGC	2.22	0.41	1.3E-03
		CAMK	2.22	0.41	1.5E-03
		TK	2.22	0.41	1.5E-03
		STE	2.22	0.41	1.4E-03

	kinase	kinase group	Enrichment score	Matthews Correlation	p-value
expressed in iCM and heart (cont.)		CAMK	2.22	0.41	1.5E-03
		AGC	2.22	0.41	1.2E-03
		CAMK	2.22	0.41	1.4E-03
		CMGC	2.22	0.41	1.4E-03
		OTHER	2.19	0.52	6.0E-05
		CAMK	2.17	0.46	3.2E-04
		CAMK	2.17	0.46	4.2E-04
		AGC	2.17	0.46	3.8E-04
		CAMK	2.14	0.50	1.0E-04
		AGC	2.12	0.42	1.8E-03
		AGC	2.12	0.42	1.9E-03
		TK	2.12	0.42	1.7E-03
		AGC	2.12	0.52	6.0E-05
		AGC	2.12	0.52	2.0E-05
		STE	2.10	0.42	1.3E-03
		OTHER	2.10	0.42	9.8E-04
		AGC	2.02	0.50	4.0E-05
expressed in heart, not in iCM		TK	2.36	0.41	1.5E-03
		AGC	2.12	0.42	1.8E-03
		TK	2.10	0.42	1.2E-03
not expressed in heart or iCM		STE	2.71	0.51	4.0E-05
		TK	2.65	0.52	4.0E-05
		STE	2.45	0.46	4.0E-05
		AGC	2.23	0.49	1.0E-04
		OTHER	2.10	0.42	1.3E-03
		CMGC	2.53	0.46	3.6E-04
		TKL	2.47	0.49	1.4E-04
		AGC	2.17	0.46	4.0E-04

Substantiation of candidate kinases contributing to functional effects by siRNA-based knockdown



A predictive model of KI cardiotoxicity by recursive partitioning analysis



Next Step

❖ Test predictive model using a test set of 110 compounds with known selectivity profiles

Conclusions

❖ KI promiscuity & phys-chem properties not adequate to predict functional cardiotoxicity

❖ Promiscuity within CAMK, OTHER, STE, and AGC kinase groups correlates with functional cardiotoxicity

❖Candidate culprit kinases of functional cardiotoxicity identified by feature importance algorithms

❖Correlates contributing to functional effects confirmed by genetic knockdown

❖ Predictive model generated by recursive partitioning analysis of a 65 compound training set to be tested with set of 150 compounds

Statistics

All data are expressed as mean and SEM of at least three independent experiments.
* indicates experimental group differs from control group by > [3*mean(SD)]

References

1. Davis et al, Comprehensive analysis of kinase inhibitor selectivity. *Nat Biotechnol.* 2011; 29(11): 1046-51