Kinase inhibitor-induced cardiotoxicity: seeking culprit kinases using cellular impedance and kinase inhibitors with kinome selectivity profiles

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Abstract

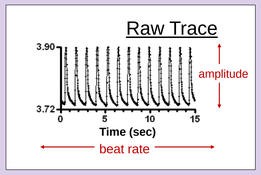
The protein kinase enzyme family has proven to be a fertile class of drug targets, with many kinase inhibitors being approved to treat a variety of clinical conditions. However, developing inhibitors of this enzyme family is not without challenges: cardiovascular (CV) toxicity is frequently observed due to off-target inhibition within this large enzyme class. Improving kinase selectivity first requires identifying the 'offending' kinase(s), i.e. requires an in vitro assay using tissue-relevant cells (preferably human) and with the throughput to screen kinome-spanning compound sets. To link kinase inhibition with CV toxicity the assay must have a functional readout that is downstream of the excitation-contraction cascade and capable of capturing the pleiotropic effects of kinases. We used real-time cellular impedance technology to quantify kinase inhibitor (KI)-induced changes in the spontaneously beating profile of human IPSC-derived cardiomyocytes (CM) cultured in 96-well plates. Effects on 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 correlated with changes in CM beating. The impedance and kinome selectivity results were interrogated using machine learning methods to identify culprit kinase profiles. Forty five kinases were identified as contributors to functional CV toxicity by recursive partitioning or feature importance algorithms. Sufficient siRNA knockdown of 30 individual kinases was achieved and confirmed effects on beating. A subset of these kinases upon knockdown in CM caused changes in action potential duration and/or calcium flux, verifying a role in CM beating and indicating distinct mechanisms of action. 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.

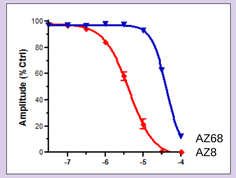
Background

- 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

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

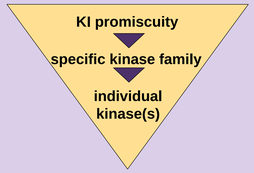




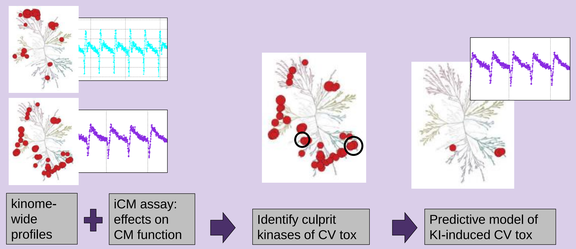
Conc-dependent inhibition of iCM beating by KIs

Strategy to test KI correlations and predict CV toxicity

1. Identify characteristics of KI that contribute to iCM beat activity



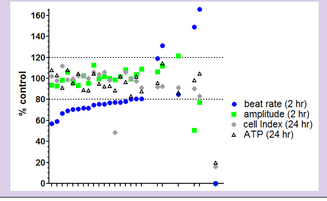
2. Develop a predictive model to identify kinases correlated with beat activity



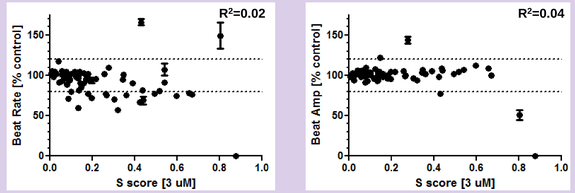
Experimental Approach

- Panel of 65 KIs whose kinome selectivity for ~385 non-redundant kinases has been published[1]
- Evaluate effects of 65 KIs on iCM beating using cellular impedance assay

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



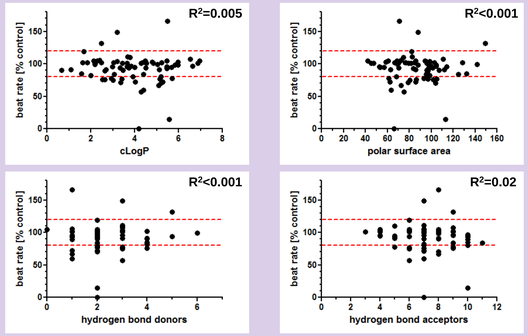
KI selectivity (S) doesn't predict iCM beating effects



Promiscuity

S score= # kinases inhibited / total kinases screened

Physico-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
OTHER	2.421	0.435
STE	2.256	0.458
AGC	2.232	0.494
Atypic	1.619	0.343
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

51% of kinome

Promiscuity within these groups correlates with beating effects

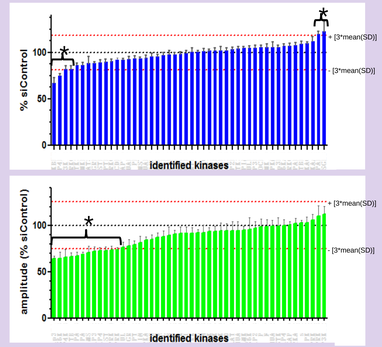
Promiscuity within these groups does not correlate with beating effects

Identification of candidate kinases (inhibition of which correlates with iCM beating effects) using feature importance algorithms

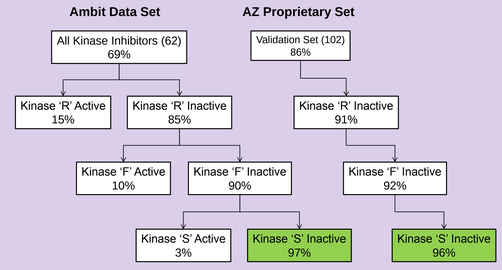
	kinase	kinase group	Enrichment score	Matthew Correlation	p-value
expressed in iCM and heart		STE	3.06	0.50	1.0E-04
		AGC	2.84	0.56	0.0E+00
		STE	2.82	0.49	1.6E-04
		AGC	2.63	0.49	2.4E-04
		TK	2.63	0.49	8.0E-05
		STE	2.55	0.42	1.2E-03
		STE	2.55	0.42	1.0E-03
		CAMK	2.54	0.56	0.0E+00
		STE	2.50	0.43	6.4E-04
		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



Compounds inactive at kinases 'R', 'F' and 'S' have 3.5% probability of inhibiting iCM beating (vs 20% of total set)

Conclusions

- **Kinase inhibitor kinome promiscuity or phys-chem properties not adequate to predict functional cardiotoxicity**
- **Promiscuity within CAMK, OTHER, STE, and AGC kinase groups correlates with functional cardiotoxicity**
- **45 candidate culprit kinases of functional cardiotoxicity identified by feature importance algorithms**
- **30 of 45 culprit kinases confirmed by siRNA knockdown**
- **Inactivity at 3 'sentinel kinases' predictive of clean profile in iCM cardiotox assay**

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