Cellular Impedance assays for deconvoluting kinase inhibitor induced cardiotoxicity

Sarah Lamore¹, Clay Scott¹, Michelle Lamb², Claudio Chuaqui², Scott Boyer¹, Johanna Sagemark¹, Lars Carlsson¹, Ernst Ahlberg¹, Jonna Stårling¹, and Matt Peters¹

¹Drug Safety and Metabolism, and ²Oncology Innovative Medicines, AstraZeneca Pharmaceuticals, Waltham, MA

Abstract

Cardiovascular (CV) toxicity is a leading contributor to drug attrition and market withdrawal. There is urgent need for faster 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 underlying kinases requires an in vitro assay with the throughput to screen kinase-relevant compound sets. Addressing CV effects requires an integrated functional assay that is downstream of the axiolation contraction cascade and capable of capturing the pleiotropic effects of kinase.

The emergence of cellular impedance technology enables detection of spontaneous beating of cultured cardiomyocytes (CM) in a real-time, label-free 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 CM. The effects on iPSC-derived CM beating were evaluated using two panels of KIs (65 and 160 compounds) whose kinase 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 requiring assays with integrated downstream endpoints to detect perturbations in their signaling pathways
• Current in vitro assays focus on arrhythmia/RERG, leaving major gaps in the evaluation of other adverse effects on contractility, beat rate and toxicity

IPS-Derived Cardiomyocyte (CM) Impedance-based Assay

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

IKM Impedance assay to test Ki correlations and predict CV toxicity

1. Identify characteristics of Ki that contribute to beat activity

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

IKM-induced changes of ICAM beat rate and amplitude at concentrations not associated with cytotoxicity

IKM selectivity (S) score is not sufficient to predict ICAM beating effects

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

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

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

Kinase inhibiting selectivity for CV toxicity

References

All data are expressed as mean and SEM of at least three independent experiments.

Statistics

All data are expressed as mean and SEM of at least three independent experiments.

Statistical analysis was performed using ANOVA followed by post-hoc Tukey’s test.

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

References