

to increase the levels of 5-HT by inhibiting monoamine oxidase (the enzyme responsible for serotonin metabolism), thereby allowing increased activation of 5-HT receptors to potentiate the HTR when compared to a negative control (saline). The objective of this study was to assess a murine model of central nervous system serotonergic activity *via* a characteristic HTR elicited following administration of carbidopa and DL-5-hydroxytryptophan (5-HTP). Additionally, two administration routes (Oral and IP) of linezolid were assessed for clinical relevance based on quantifiable HTR. Consistency between dose routes would permit greater flexibility of preclinical study designs, allowing for a comparison to intended routes of administration and anticipated exposure in humans. Head twitches were recorded for at least 20 min (15–35 min) following administration of different control articles in male mice to evaluate the potential HTR. Our results show HTRs were appropriately potentiated by administration of the positive control selected. The comparison of oral and IP dosing of linezolid exhibited similar head twitch potentiation results. These results suggest the allowance of multiple routes of comparison for clinical relevance, thereby potentially closing the gap between preclinical and clinical data to derisk serotonin toxicity.

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Phenotypic profiling for understanding mechanisms of cardiovascular adverse reactions

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Cardiovascular toxicities can arise from diverse mechanisms due to untoward drug effects causing either arrhythmia, hypertension or structural remodeling. The situation with tyrosine kinase inhibitor (TKI) oncology drugs is further complicated by the number of targets with which these drugs interact. Understanding these different mechanisms and the role of different kinases in cardiotoxicity-related processes may be helpful in mitigating cardiovascular risk. We have previously shown that data mining a large reference database containing drugs, experimental chemicals, and other agents profiled in a panel of human primary cell-based systems (BioMAP® Systems) can discover novel clinical associations and mechanisms of toxicity. We have previously described mechanisms for skin rash and thrombosis-related side effects. Using this approach an *in vitro* signature for cardiovascular toxicity was discovered. This signature is comprised of increased cell surface levels of serum amyloid A (SAA) measured in a coronary artery smooth muscle cell-based model of vascular inflammation (BioMAP CASM3C system) and found to be associated with MEK, HDAC, GR and IL-6R mechanisms. To discover mechanisms with potential for cardioprotection, we performed data mining in the same assay to identify agents that reduce the level of SAA. Thirty-two of 3800 reference agents tested (<1%) were found with this activity. Active agents include GLP-1, an endogenous peptide and diabetes drug, roflumilast, a PDE IV inhibitor and treatment for lung disease, imatinib, a BCR-Abl inhibitor and oncology drug, and a mimetic of ApoA-1, the major lipoprotein of HDL. These agents have been shown to have cardiovascular protective effects in clinical or *in vivo* studies. In addition, five experimental non-selective TKIs of AKT, p38 MAPK, MEK, and VEGR2, were also found to reduce SAA levels. The diversity of these targets and lack of activity by more selective compounds, suggests that the effect on SAA may be due to a common

off-target. This assay may be useful for assessing next generation TKIs for potential cardiovascular effects in humans.

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The mast cell receptor MrgX2 and drug-induced anaphylaxis

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Activation of MrgX2, a G-protein-coupled receptor expressed on mast cells, leads to degranulation and histamine release. MrgX2 (also known as MRGPRX2) binds to basic peptides and small molecules, resulting in acute histamine-like reactions to drugs, both in man, and in mammalian species used in drug testing for toxicology. Here, we investigate the *in vitro* pharmacology of both human MrgX2 and orthologous receptors identified from mouse, rat, dog (Boxer and Beagle), minipig, pig, and Rhesus monkey. We consider whether new chemical entities considered for clinical development commonly activate MrgX2, and whether testing for MrgX2 agonism during optimisation of drug leads is justified. MrgX2 was introduced into cultured human U2OS cells by baculovirus-mediated transduction. Agonist-evoked intracellular calcium release was measured using fluorescence-imaging plate readers (FLIPR), after loading cells with calcium-sensitive dyes. Confirmatory results were obtained by quantifying agonist-induced association of MrgX2 with the intracellular adaptor protein, beta-arrestin. We present a series of case studies in which small-molecule and peptide agents, that previously caused unexplained anaphylaxis in preclinical *in vivo* tests, were confirmed to be MrgX2 agonists. Screening further small-molecule and peptide agents underlined the extraordinary promiscuity of MrgX2: *all* peptides tested having net charge +3 or greater (high basicity) exhibited agonist activity. This included cell penetrating peptides polyarginine (acetyl-Arg₉-amide) and TAT(49–60), a fragment of HIV-1 TAT protein. Our results illustrate how knowledge of MrgX2 activity across this panel of mammalian species can be integrated during drug development to influence choice of compound, species model and dosing regimen. Insight into MrgX2 structure-activity relationship (SAR) offers clues as to the physiological role of this receptor.

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Simultaneous integration of telemetered left-ventricular pressure and echocardiography in rats: Minimally-invasive serial pressure-volume relationships in the setting of normal and impaired diastolic function

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