Reliable and Predictive In Vitro Assays for Myelotoxicity and Cardiotoxicity of Kinase Inhibitors

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INTRODUCTION

Kinase inhibitors (KIs) represent a new class of rationally designed drugs. The success of Imatinib, targeting the ABL tyrosine kinase in CML, has prompted the development of other KIs for the treatment of various cancers and inflammation. Although more successful than conventional therapies, myelotoxicity and cardiotoxicity are often major side effects of KIs. In order to predict if newly developed molecules demonstrated significant myelotoxicity or cardiotoxicity, we assessed a number of TKIs using in vitro models. To assess myelotoxicity, a human bone marrow progenitor assay (colony forming cell or CFC assay) previously validated by ECVAM was used. To assess cardiotoxicity, mouse ES cell-derived cardiomyocytes were used as a primary-like cellular platform in an in vitro cytotoxicity assay.

MATERIALS AND METHODS

For the in vitro myelotoxicity assay, clonogenic progenitors of the human myeloid (CFU-GM) lineage were set up in the methylcellulose-based media formulation (R&D Systems, MN). Six KIs were selected for testing: Imatinib, Lapatinib, Erlotinib, Dasatinib, Sorafenib and Sunitinib. These were chosen based upon their different target and disease specifications (*Table 1*) as well as reported differences in their clinical toxicity profiles. The KIs were added to the medium to give final concentrations ranging from 100 to 0.001 µg/mL. Solvent control cultures were also initiated. The cultures were set up in triplicate with normal pre-qualified human bone marrow (ReachBio, WA) from three different donors. Following incubation at 37ºC, 5% CO_2, for 14-16 days, the colonies were assessed and scored by trained personnel.

For the in vitro cardiotoxicity assay, mouse ES cell-derived cardiomyocytes (Cor.AT® cells, Axiogenesis, Germany) were plated in 24-well plates at 20,000 cells per well (6 replicates per condition) in Cor.At culture medium. The cells were cultured for 14 days so as to obtain a mature phenotype. The KIs were then added at concentrations ranging from $10^{-4} - 10^{-9}$ M. Cell death was measured by neutral red uptake 48 hours after the addition of KIs. The KIs were also added for the same amount of time and at the same concentrations used with the cardiomyocyte cultures to cultures of mouse embryonic fibroblasts (used as non-specific control cells), and fibroblast cell death was assessed by neutral red uptake.

TABLE 1 Target and Disease Specifications

TKI	TARGET	TREATMENT
Imatinib	ABL/ PDGF/ KIT	CML and Ph+ B-ALL
Dasatinib	ABL/ PDGF/ KIT/ src	CML
Sorafenib	VEGFR2/ KIT/ PDGF/ RAF/FLt3	Renal cel carcinoma/ myeloma
Sunitinib	VEGFR1-3/ KIT/ PDGF/ CSFR1/FLt3	Renal cell carcinoma
Lapatinib	ERB/ EGFR	Breast Cancer
Erlotinib	RGFR/mutant JAK2 kinase	Lung Caner and possibly PV, MF

RESULTS

Rank order of myelotoxiciity potential in vitro correlates with published clinical observations.

The IC_{50} values derived from the in vitro CFC assays allowed us to rank the CFCs in terms of myelotoxicity (*Figure 2*). Remarkably, there was a direct correlation between the reported clinical myelotoxicity and the IC_{50} values derived from the CFC assays, with lower IC values associated with increased neutropenia (*Table 2, Figure 1*). Dasatinib was the most toxic KI (IC_{50} value: 0.008 ug/mL) and Lapatinib was the least toxic (IC_{50} value > 100 ug/mL), *Table 2, Figure 2*).

In vitro cardiotoxicity assay results and clinical observations closely associated.

There was also a strong association between published clinical cardiotoxicity (e.g. myopericarditis, ischemia, pericarditis, myocardial infarction) and the in vitro effect of the KIs on mature (day 14) cultures of cardiomyocytes derived from mouse ES cells (Cor.At® cells) (*Table 3*). Sorafenib was the most toxic KI where as Erlotinib and Lapatinib demonstrated the least toxicity (*Table 3 and Figure 3*). The profile of toxicity of the KIs on mouse ES-derived cardiomyocytes was not the same as that from mouse embryonic fibroblasts, suggesting that specific cardiomyocyte toxicity could be distinguished from a more generalized cytotoxic effects in these assays (*Table 3*).

FIGURE 1 Correlation between CFU-GM IC_{50} Values and Clinical Neutropenia for Kinase Inhibitors

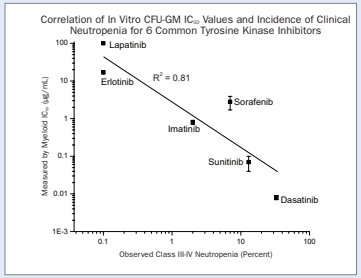


FIGURE 2 Kinase Inhibitor Myelotoxicity may be Ranked, based on CFU-GM Assay

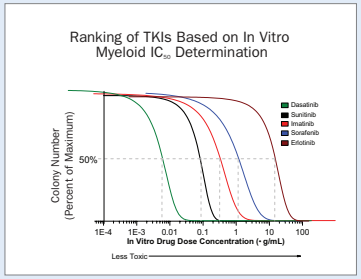


TABLE 2 In Vitro CFU-GM IC_{50} Values Correlate with Clinical Neutropenia

	TOXICITY RANKING	IC_{50} CFU-GM	NEUTROPENIA GRADE 1/11	NEUTROPENIA GRADE 111/1V	REFERENCE
Dasatinib	1	0.008 ug/mL	30%	33%	J Clin Oncol 26 (19): 3204-3212, 2008
Sunitinib	2	0.09 ug/mL	32	13%	J Clin Oncol 24 (1): 16-24, 2006
Imatinib	3	2.6 ug/mL	6%	2%	J Clin Oncol 22 (1): 77-85, 2004
Sorafenib	4	3.5 ug/mL	ND	7%	J Clin Oncol 25 (24): 3766-3773, 2006
Erlotinib	5	16 ug/mL	ND	ND	J Clin Oncol 26 (4): 563-569, 2008
Lapatinib	6	> 100 ug/mL	ND	ND	The Oncologist 12 : 756-765, 2007

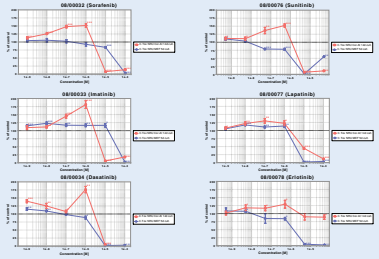
ND= none detected

TABLE 3 IC_{50} Values from an In Vitro Cardiomyocyte (Cor AT®) Toxicity Assay is Associated with Clinical Cardiotoxicity

COLUMN 1	IC_{50} NEUTRAL RED UPTAKE ES-DERIVED CARDIOMYOCYTES	IC_{50} NEUTRAL RED UPTAKE MOUSE EMBRYONIC FIBROBLASTS	RANKING COR.AT /MEF	CARDIOTOXICITY IN VITRO	REFERENCE
Sorafenib	0.229 µg/mL	12.6 µg/ml	1	++	J Clin Oncol 24(9): 1363-1369, 2006
Imatinib	0.442 µg/mL	18.6 µg/ml	2	++	Nat Med 12(8): 908-916, 2006
Dasatinib	0.203 µg/mL	4.54 µg/ml	3	++	
Sunitinib	0.142 µg/mL	0.465 µg/ml	4	+	Mol Pharmacol 74 (6): 1722-1728, 2008;
Lapatinib	4.97 µg/mL	2.16 µg/ml	5	/	The Oncologist 12:756-765, 2007
Erlotinib	ND	0.528 µg/ml	6	/	NA (only reports on skin and lung toxicity)

ND= none detected

FIGURE 3 Comparison of Toxicity Profiles for Kinase Inhibitors in Cor.At® Cardiomyocyes and Mouse Embryonic Fibroblasts



CONCLUSION

- There is a direct correlation ($r^2 = 0.81$) between the in vitro human CFU-GM IC_{50} values for various kinase inhibitors and clinical neutropenia.
- The human CFU-GM assay can be used to rank KIs in terms of myelotoxicity potential as has been shown previously with certain other compound classes.
- The human CFU-GM assay could be used to compare myelotoxicity between various classes of compounds and predict the myelotoxicity potential of new compounds.
- There is an association between the results of the in vitro cytototoxicity assays using mouse ES-derived cardiomyocytes (Cor AT®) and clinical cardiotoxicity. Work is ongoing to optimize this assay on a number of different parameters and additional tests are being performed to study specific mechanisms of the toxicity in vitro (e.g., mitochondrial dysfunction).

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