

Differential Toxicities of Kinase Inhibitors (KI) on Bone Marrow Progenitors from Different Species

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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 is often a major side effect of KIs. We previously showed that there was a direct correlation ($R^2 = 0.81$) between the in vitro human CFU-GM IC_{50} values for various KIs and clinical neutropenia. Interestingly, studies have revealed significant differences between human, dog, rat and mouse CFU-GM sensitivities to certain pharmaceuticals. Since multiple animal models are often used in toxicity testing, we have now compared the IC_{50} values between human, non-human primate, dog, rat and mouse for Imatinib, Erlotinib, Dasatinib, Sorafenib and Sunitinib.

MATERIALS AND METHODS

Based on our previous data set generated using human bone marrow (Figure 1), five KIs were selected for testing: Imatinib, Erlotinib, Dasatinib, Sorafenib and Sunitinib. These KIs have different target and disease specifications (Table 1) and also have reported differences in their clinical toxicity profiles. Clonogenic progenitor assays of the human, non-human primate, dog, rat and mouse myeloid (CFU-GM) lineage were set up in ColonyGEL[®] methylcellulose-based media formulations optimized for each species with appropriate species-specific cytokines (ReachBio, WA). The KIs were added to the various media formulations to give final concentrations ranging from 100 to 0.0001 μ M. Solvent control cultures were also initiated. The cultures were set up in triplicate 35 mm dishes with cells derived from normal human bone marrow (3×10^7 /culture), non-human primate bone marrow from both rhesus and macaque (3×10^7 /culture), dog bone marrow (2×10^5 /culture), rat femoral bone marrow (5×10^4 /culture) and mouse femoral bone marrow (2×10^7 /culture). The experiment was set up using cells from 3 individual donors/species. Following incubation at 37°C, 5% CO₂, for 7-16 days (depending on the species), the colonies were assessed and scored by trained personnel and IC_{50} values were determined for each drug for each species. Representative photos of the CFU-GM derived colonies of each species are presented in Figure 3.

TABLE 1 Target and Disease Specifications of Kinase Inhibitors Tested

TKI	Target	Treatment
Imatinib	ABL/ PDGF/ KIT	CML
Dasatinib	ABL/ PDGF/ KIT/ src	CML
Sorafenib	VEGFR2/ KIT/ PDGF/ RAF/FL13	Renal cell carcinoma/ myeloma
Sunitinib	VEGFR1-3/ KIT/ PDGF/ CSFR1/FL13	Renal cell carcinoma
Erlotinib	RGFR/mutant JAK2 kinase	Lung Cancer and possibly PV and MF

RESULTS

IC_{50} values determined for the 5 KIs using non-human primate and dog marrow compared well with human values (Table 2, Figure 2). There was no difference in the non-human primate IC_{50} values, whether the marrow was derived from a rhesus or a macaque monkey. The rank order of in vitro toxicity of the 5 KIs was also the same in human, non-human primate and dog CFU-GM assays, mirroring their human clinical myelosuppression rankings. In contrast, the IC_{50} values for the same drugs in rat and mouse CFU-GM assays differed significantly from human values, with over a log difference in IC_{50} values for Imatinib and Dasatinib (Table 2). When tested with mouse and rat bone marrow, the KIs also did not rank in the same order as they did in human, non-human primate and dog assays (Table 2, Figure 2), or as they do with respect to their reported human clinical myelosuppressive levels.

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

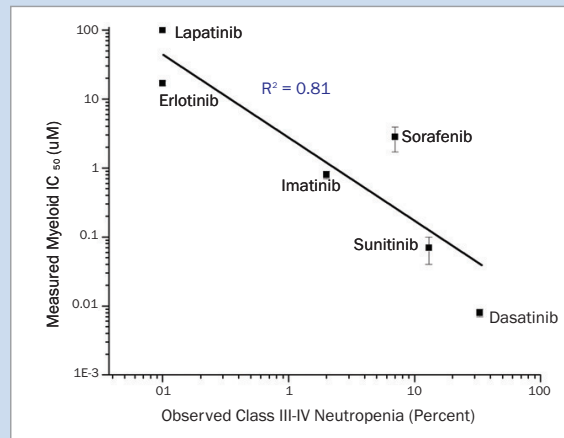


FIGURE 2 Comparison of CFU-GM IC_{50} Values for TKIs from Different Species

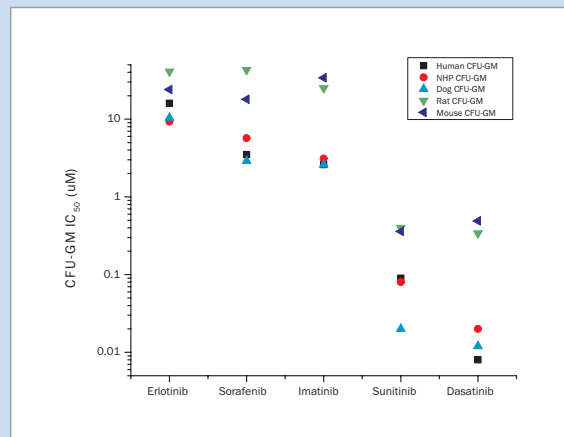
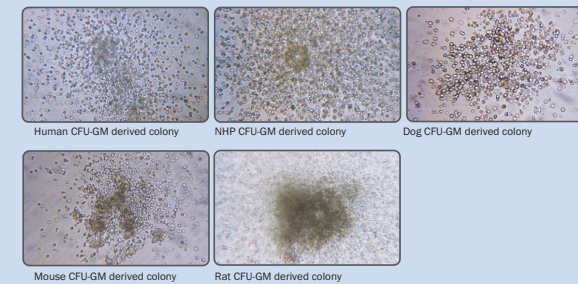


TABLE 2 CFU-GM IC_{50} Values for Kinase Inhibitors in Different Species

	Human CFU-GM IC_{50} (uM)	NHP CFU-GM IC_{50} (uM)	Dog CFU-GM IC_{50} (uM)	Rat CFU-GM IC_{50} (uM)	Mouse CFU-GM IC_{50} (uM)
Erlotinib	1.6	9.3	10.4	41	24
Sorafenib	3.5	5.7	2.9	43	18
Imatinib	2.6	3.1	2.6	25	34
Sunitinib	0.09	0.08	0.02	0.4	0.36
Dasatinib	0.008	0.02	0.012	0.34	0.49

FIGURE 3 Representative Photos of CFU-GM Derived Colonies from Various Species



CONCLUSIONS

- The IC_{50} values of the 5 KIs tested in the non-human primate and dog marrow CFU-GM assays compared well with human CFU-GM IC_{50} values.
- The rank order of the compounds in terms of toxicity (as measured by the CFU-GM IC_{50} value) was the same for human, non-human primate and dog, and corresponded with their rankings in terms of human clinical myelotoxic potential.
- The IC_{50} values for the KIs in mouse and rat CFU-GM assays differed significantly from those obtained in human CFU-GM assays with values greater than a log difference for Imatinib and Dasatinib
- The rank order of toxicity of the KIs in mouse and rat CFU-GM assays was different to their order in the human CFU-GM assay and in the clinic.
- It is possible that data generated from rat and mouse assays for KIs may not as accurately predict the toxicity of these compounds on the human hematopoietic system as data from human, non-human primate or dog assays.

