Supplementary Material

Bortezomib plasma concentration detection assay

Plasma samples for PK analyses were thawed in an ice bath. 200 µL of each sample was mixed with 100 µL of internal standard solution [10 ng/mL of [$^{13}C_9$] bortezomib in acetonitrile:water containing 0.1% formic acid (55:45, v:v)], 100 µL of 100 mM ammonium formate (pH 3.0) and 1000 µL of methyl-tert-butyl ether. The sample was shaken for approximately 10 minutes followed by centrifuging at 3,000 rpm for approximately 10 minutes. Samples were then frozen in a methanol/dry ice bath, and the organic layer transferred from each tube into a clean polypropylene test tube. Samples were then evaporated under nitrogen at 40°C, reconstituted by adding 150 µL of acetonitrile:water containing 0.1% formic acid (55:45, v:v), vortexed for approximately 1 minute, and transferred into a 96 well plate. The plate was then capped and placed into an autosampler set at room temperature. Approximately 10 µL of the sample solution were loaded onto a Sunfire C8, 2.1 mm internal diameter x 50 mm, 5 µm HPLC column (Waters, Milford, MA) set at 60°C and separated by gradient elution using a mixture of acetonitrile, water containing 0.1% formic acid, and 25 mM ammonium acetate in isopropanol at a flow rate of 0.4 mL/min. The run time was approximately 7.5 minutes. A Sciex API 4000 LC/MS/MS system with a turbo ionspray interface (Concord, ON, Canada), operated with a source temperature of 550°C, was used for detection. Multiple reaction monitoring with a dwell time of 200 msec was used to detect positive ions resulting from the *m/z* 367.1→226.0 transitions for bortezomib and 50 msec was used to detect positive ions resulting from the *m/z* 376.1→243.0 transitions for the internal standard. Quantitation was based upon integrating peaks corresponding to

elution of the drug and internal standard in the extracted product ion chromatograms. The concentration range of the calibration standards of bortezomib in human plasma was 0.100 to 25.0 ng/mL. Study samples were assayed together with a series of 7 calibration standards and at least 8 quality control samples distributed at four levels over the curve range. Standard curves were constructed by plotting the drug/internal standard chromatographic peak area ratio against the known drug concentration in each calibration standard. Linear least squares regression was performed with weighting in proportion to concentration ($1/x^2$) of each calibration standard. Values of the slope and *y*-intercept of the best-fit line were used to calculate the drug concentration in study samples. Specimens with concentrations exceeding the upper range of the standard curve were re-assayed upon appropriate dilution with drug-free human plasma.