Bortezomib (PS-341, VELCADE®) is a novel chemical entity that potently and specifically inhibits the proteolytic activity of the proteasome and thus the degradation of poly-ubiquitinated proteins destined for catalysis by the proteasome. Proteasome mediated degradation has been shown to be a highly regulated process involved in the degradation of misfolded and senescent intracellular proteins as well as in the normal homeostasis of many intracellular proteins, and thus it plays a fundamental role in normal cell biology [1]. Numerous proteins involved in cell cycle control and apoptosis have been shown to be regulated by proteasome degradation. The net consequence of proteasome inhibition in a variety of cancer cells is induction of apoptosis, and this observation has provided a basis for the development of bortezomib as an anticancer agent [2]. Bortezomib is the first proteasome inhibitor to be developed and successfully brought to market as an anticancer agent. A customized nonclinical development program was devised to support the clinical development and registration of bortezomib for the treatment of multiple myeloma (MM). This brief summary outlines the objectives and principal findings of the nonclinical program.

The objectives of the pharmacology program for bortezomib were to establish the potency and specificity for the proteasome, establish anti-cancer activity in vitro and in vivo, develop an understanding of the mechanism of anti-cancer activity in MM, study the potential for tumor resistance, optimize the regimen of administration and establish a monitorable biomarker of effect.

Bortezomib is a potent (0.6 nM Ki) and reversible inhibitor of the proteasome and shows no significant inhibitory activity against other related or unrelated enzymes or receptors. Bortezomib displays a potent and novel pattern of cytotoxicity in the NCI cancer cell screen. It also shows a high degree of cytotoxic selectivity for cancer cells as compared with non-cancerous cells (10- to 1000-fold). The mechanism of cytotoxicity of bortezomib is unique as compared with most classical cytotoxic agents. In addition to direct cytotoxicity, bortezomib modulates the bone marrow microenvironment in several ways to inhibit growth of MM cells. In murine xenograft models bortezomib has shown dosage-related anti-tumor activity in multiple myeloma and a variety of other cancers, both as a single agent or when combined with other cytotoxic therapies. Treatment emergent tumor resistance has not been seen in preclinical models and multi-drug resistance (MDR) expressing tumor cells or tumors that express a number of tumor virulence conferring mutations are not resistant to tumor killing with bortezomib. A biomarker of proteasome inhibition (20S proteasome catalytic activity) was established and the proteasome activity as measured in peripheral blood is reflective of activity within tumor xenografts or other distant tissues. The twice weekly regimen of administration was developed considering the duration of pharmacodynamic (PD) effect (approximately 72 hours), the demonstration that maximum tolerated dosage produces maximal anti-tumor activity, and tolerability studies showing that dosage intensity over time is maximized when proteasome activity is allowed to return toward baseline between doses.

The objectives of the drug metabolism and pharmacokinetic (PK) program were to measure exposure and PD effects of bortezomib after intravenous (IV) administration, characterize the distribution within the body, identify the metabolites, determine the rates and routes of elimination from the body, and predict the potential for drug-drug interactions.
After single dose IV administration to rats and cynomolgus monkeys, plasma concentrations of bortezomib declined in a bi-phasic manner with a rapid distribution phase followed by a longer terminal elimination phase. The elimination half-life in cynomolgus monkeys averaged 8 to 10 hours. The area under the plasma concentration-time curve (AUC) increased in a dose-dependent manner. After multiple doses of bortezomib, the elimination half-life in cynomolgus monkeys increased 3- to 4-fold. The increase in half-life observed in cynomolgus monkeys was paralleled by a decrease in total body clearance resulting in a several fold increase in the AUC. The relationship between drug concentration and the pharmacodynamic effects of proteasome inhibition has been well established across several species. For example, in cancer patients, the PK/PD relationship is described by a simple E$_{max}$ model with a plasma EC$_{50}$ of 1.48 ng/mL. A steep portion of the concentration-response curve up to 2 ng/mL was followed by a plateau at approximately 70 - 80% inhibition, when even doubling or tripling of the concentration resulted in a marginal increase in 20S proteasome inhibition. Therefore, while the relationship between the 20S activity assay and plasma concentration shows a narrow dynamic range, it has been useful to define concentrations of bortezomib in the pharmacologically active concentration range.

Tissue distribution studies in the rat and monkey have shown that after IV administration, there was rapid movement of radioactivity from the vascular compartment into tissues except for the central nervous system and testis. Most tissues had tissue/blood concentration ratios of greater than one and studies in xenograft models in the mouse also indicated uptake into tumors. Bortezomib and its radiolabelled metabolites are slowly eliminated from the body. Biliary excretion is the primary route of elimination of [14C]-bortezomib derived radioactivity in rats, and [-C-bortezomib-derived radioactivity was excreted in both the urine and bile in the monkey.

Bortezomib is extensively metabolized in rats, cynomolgus monkeys, and humans. More than thirty different in vitro and in vivo metabolites have been identified. Qualitatively, the metabolism of bortezomib is similar in rats, cynomolgus monkeys and humans. In vitro and in vivo studies indicated that bortezomib is primarily metabolized via cytochrome (CYP) P450. Studies using human recombinant expressed CYP 450 isozymes indicated that bortezomib was metabolized fastest by CYP 3A4 and to a lesser extent by CYPs 2C19 and 2D6. The major in vitro and in vivo bortezomib metabolic pathway is deboronation, and deboronated metabolites have been shown to be pharmacologically inactive against 20S proteasome activity. Bortezomib is a poor inhibitor of human recombinant expressed CYP 450 enzymes. Overall, it is considered that bortezomib has a low potential for drug-drug interactions.

The objectives of the preclinical toxicity program were to establish the potential for genotoxic activity and developmental toxicity, establish transpecies toxicity dose-response information in the most relevant species, establish the target organs of toxicity, determine the reversibility of toxicity, establish relationships of toxicity to exposure and PD, examine the potential for important toxicologic drug interactions, understand the mechanism of potentially important toxicities, and to establish the pharmacologic safety on critical organ systems.

Genotoxicity testing has shown that bortezomib is non-mutagenic in the Ames bacterial mutagenicity assay and negative for chromosomal damaging effects in the in vivo micronucleus assay. However, bortezomib was positive for induction of structural chromosomal aberrations in the in vitro chromosomal aberrations assay. This finding was expected based on the known cell cycle effects of bortezomib and is considered an extension of the pharmacological activity, and is an acceptable characteristic of an anti-cancer drug. Developmental toxicity studies in the rat and rabbit have shown embryo-fetal lethality at maternally toxic dosages, but no direct embryo-fetal toxicity below maternally toxic dosages.

Single-dose IV toxicity studies were conducted with bortezomib in the mouse, rat, dog and monkey to establish the single dose maximum tolerated dose (MTD). The Sprague-Dawley rat and cynomolgus monkey were the most sensitive toxicology test species and were studied for the repeated-dose IV toxicity studies.

Pivotal repeat-dose multi-cycle toxicity studies of 6 months in the rat and 9 months in the monkey, each with 8-week recovery periods, were conducted to characterize the chronic toxicity of bortezomib when administered by the clinical route and regimen of administration (3 week cycles with twice weekly IV dose administration for two weeks followed by one week rest). The MTD in the 6-month rat study was 0.10 mg/kg (0.6 mg/m2) and the key target organs were the gastrointestinal tract and the hematopoietic and lymphoid systems. The MTD in the 9-month monkey study was 0.05 mg/kg (0.6 mg/m2) and the key target organs were the gastrointestinal tract, hematopoietic and lymphoid systems, peripheral nervous system (PNS), and kidneys. Full or partial reversibility has been observed for each of the toxicities described. In general, the nature of the toxicity of bortezomib is similar across species, and target organs of toxicity in animals have been largely predictive of human toxicity. The dosages used in the pivotal repeat-dose studies are comparable to the clinical dosage of 1.3 mg/m2.
The toxicity of bortezomib in animals is characterized by a steep dose-response with mortality seen at dosages above the MTD. The cause of death at acutely lethal dosages is considered to be related to the cardiovascular effects of hypotension with initial tachycardia followed by progressive bradycardia and ultimately cardiovascular collapse. The cause of death in long term studies has been attributed to either gastrointestinal or hematologic toxicity. Overall, the nonclinical safety profile of bortezomib is comparable in many ways to other cytotoxic anticancer agents showing a narrow safety margin, but with well defined and an easily monitorable safety profile. The nonclinical pharmacology, DMPK and toxicology of bortezomib have been well described. The observed efficacy, drug disposition, and toxicity profile in clinical trials has mirrored the nonclinical data very closely. Bortezomib has been shown to be both safe and efficacious in the treatment of MM and was recently approved for this use in the United States and Europe [3]. Because of the apparent broad role of the proteasome in cancer cell biology, bortezomib is being evaluated in further clinical indications and will hopefully prove to be effective in the treatment of other cancers.

References


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