In this issue, Zou et al. report that vitamin C abrogated the ability of bortezomib (PS-341, Velcade) to induce apoptosis, G2-M arrest, and cleavage of caspase 8, caspase 3, and poly(ADP-ribose) polymerase and to augment tumor necrosis factor–related apoptosis-inducing ligand–induced apoptosis in various human cancer cell lines (1). Moreover, vitamin C suppressed bortezomib-mediated inhibition of proteasome activity. Bortezomib did not induce generation of intracellular reactive oxygen species and other antioxidants failed to abrogate its biological activity. Accordingly, the data suggest that vitamin C protects cells from bortezomib-induced apoptosis independent of its antioxidant activity. The suppression of bortezomib-induced cell death was not cell line specific, although concentrations required for bortezomib inhibition varied in different cell lines. The evidence presented is consistent with a direct chemical interaction between vitamin C and bortezomib. Vitamin C exerts its protective effect on bortezomib-induced apoptosis only when it is added concurrently and by directly binding to bortezomib, thereby abrogating its suppressive effects on 20S proteasome activity. This interaction may inhibit intracellular transport, entry into the central chamber of the proteasome, or binding of bortezomib to the catalytic sites of the proteasome.

Vitamin C has previously been proposed as a tonic against many diverse illnesses, from the common cold to cancer; however, supporting data for such benefits are incomplete and sometimes contradictory. Vitamin C has been reported to have protective cellular effects via its antioxidant properties and toxic effects via generation of reactive oxygen species and depletion of cellular glutathione. Furthermore, the production of reactive oxygen species in vitro by vitamin C may occur only in tissue culture due to the presence of free transition metal ions, such as iron and copper, in culture media. In vivo, these metal ions are bound by transition metal–binding proteins, such as ferritin.

In vitro, vitamin C is toxic to a variety of cancer cell lines: extracellular concentrations as low as 100 to 200 μmol/L are toxic to some cell lines but many types of malignant cells are killed only at concentrations approaching the millimolar range. These high plasma concentrations are achieved only via i.v., but not oral, administration of vitamin C. The combination of arsenic trioxide (As2O3) with the reduced form of vitamin C, ascorbic acid, has shown synergistic activity against multiple myeloma both in vitro and in vivo (2, 3), and the combination has shown activity against relapsed and refractory multiple myeloma in clinical trials (4). As2O3 with ascorbic acid is now being evaluated in combination with either melphalan or dexamethasone in separate phase II studies in the United States. As2O3 induces superoxide production while reducing glutathione levels, resulting in mitochondrial-mediated apoptosis; 100 μmol/L ascorbic acid alone has little effect on multiple myeloma cells but decreases glutathione levels and thereby potentiates As2O3-mediated multiple myeloma cell death (2). The primary determinant of multiple myeloma cell sensitivity to As2O3 seems to be intracellular glutathione levels because elevated glutathione levels are associated with chemoresistance. In chemosensitive as well as refractory multiple myeloma cells, ascorbic acid depletes intracellular glutathione, increases hydrogen peroxide production, and potentiates As2O3-mediated disruption of mitochondrial membrane potential. Conversely, vitamin C protects HL60 leukemia cells and U266 multiple myeloma cells from As2O3 toxicity by decreasing intracellular reactive oxygen species (5). These paradoxical effects have been associated with differences in intracellular vitamin C concentrations.

When considering the effect of these data on patient care, the biological functions and pharmacokinetics of vitamin C are an important consideration. Vitamin C is vital for collagen production and bile acid synthesis and aids in iron absorption. Most animals synthesize their own vitamin C within their liver, with the exception of fish, primates, and humans. Good sources of vitamin C in nature include fresh fruits and vegetables. Vitamin C is a water-soluble vitamin commonly added as an antioxidant to protect color and aroma of food. Daily intake of 100 mg of vitamin C will prevent scurvy for 1 month. On a stable diet, human plasma levels of vitamin C range from 50 to 70 μmol/L as doses exceed 200 mg; its absorption decreases, urinary excretion increases, and bioavailability is reduced (6).

Plasma vitamin C concentration is completely saturated at oral doses of ≥400 mg daily, with a steady-state fasting plasma concentration of 80 to 100 μmol/L (7, 8). At concentrations ranging from 62.5 to 500 μmol/L, vitamin C abrogates the ability of bortezomib to induce apoptosis and growth arrest with a vitamin C to bortezomib binding constant of 645 μmol/L, close to the concentration of vitamin C (i.e., 500 μmol/L) that substantially inactivates bortezomib in cells. In cancer treatment, vitamin C at a dose of 1.25 g administered orally or intravenously produces mean peak plasma concentrations of ~135 or 900 μmol/L, respectively (7, 9). Therefore, interactions between vitamin C and bortezomib may occur in the setting of simultaneous vitamin C administration only at supraphysiologic concentrations. Although the strongest inhibition of bortezomib by vitamin C was seen at 500 μmol/L, some inhibition was also observed at 125 μmol/L.

Because the mechanism of vitamin C–induced inhibition is via direct binding to bortezomib, it is likely that these effects also occur in normal cells, with implications for its therapeutic index. Significant toxicities to normal tissue from bortezomib...
therapy include peripheral neuropathy, diarrhea, fatigue, and myelosuppression. Bortezomib toxicity may be a consequence of proteasome inhibition with subsequent interference with multiple cellular proteins in normal cells; however, the ultimate causes of bortezomib-related side effects remain undefined and the subject of ongoing clinical and laboratory research. The binding of vitamin C to bortezomib may inhibit many downstream effects, including intended tumor cytotoxicity as well as unintended side effects in normal tissues. Due to oxidative stress, tumor tissues may have relatively low levels of vitamin C whereas higher concentrations of vitamin C in normal tissues or organs, such as skeletal and smooth muscle, leukocytes, brain, adrenal glands, and lung, may protect them from bortezomib toxicity. For example, vitamin C concentrations in circulating neutrophils, lymphocytes, and monocytes reach 1 to 4 mmol/L at vitamin C doses between 100 and 200 mg daily (7, 8). Therefore, a regular healthy diet of fresh fruits and vegetables would maintain tissue vitamin C levels sufficient to alleviate some of the toxicity of bortezomib but not sufficient to prevent entry of bortezomib into malignant cells and its cytotoxic activity. These data highlight the need for further studies on the metabolism of bortezomib and vitamin C in normal tissues in patients undergoing treatment with bortezomib to determine the potential effect of intracellular vitamin C on drug resistance and unintended toxicity.

Finally, there remains the possibility that novel therapeutic strategies may result in interactions between i.v. vitamin C and bortezomib. For example, current treatment strategies in multiple myeloma include \( \text{As}_2\text{O}_3 \) in combination with i.v. vitamin C; future clinical trials combining bortezomib plus \( \text{As}_2\text{O}_3 \) may include a schedule and/or dose of i.v. vitamin C designed to augment the activity \( \text{As}_2\text{O}_3 \) but which may also interfere with bortezomib (10). Therefore, further confirmation of the data presented by Zou et al, coupled with an increased understanding of the metabolism of vitamin C in normal versus malignant tissue, will be required to provide a platform for overcoming drug resistance in malignant cells and reduce toxicity in normal tissue.

References
Velcade and Vitamin C: Too Much of a Good Thing?
Laurence Catley and Kenneth C. Anderson