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Novel proteasome inhibitors

The ubiquitin proteasome pathway plays a central role in controlling intracellular turnover of proteins regulating cell growth and survival, stress responses, apoptosis and cell cycle.[1] Proteasome inhibitors were initially developed in order to investigate the mechanisms of cellular proteolysis; early studies, however, showed that these compounds induced apoptosis preferentially in tumor cells and were thus considered antineoplastic drug candidates.[2] The 20s core of the proteasome contains three major sites of proteolytic activity, including a chymotrypsin-like activity, a trypsin -like activity and a post-glutamyl peptide hydrolyzing or caspase-like activity. Bortezomib is the first proteasome inhibitor that shown effective in the treatment of haematological malignancies such as multiple myeloma (MM), and mantle cell lymphoma.[3-4] This compound is a boronic acid derivative that inhibits proteasoma activity by reversible binding to the chymotrypsin-like site of the 20s subunit.[5] In order to further exploit proteasome inhibitors as antineoplastic agents, other synthetic or natural compounds were studied *in vitro* or in animal models.

NPI-0052 is a small molecule derived from fermentation of Salinospora, a gram-positive actinomycete.[6] This drug, as bortezomib, inhibits chymotrypsin-like site of the 20s subunit of the proteasome, but, in addition, is able to reduce trypsin-like and caspase-like activities; all these effects seem to be more prolonged than those observed using bortezomib.[7] NPI-0052 has been demonstrated to induce apoptosis in MM cells resistant to chemotherapy or bortezomib, and to prolong survival in a human plasmacytoma xenograft mouse model; the mechanism of action is different from that displayed by bortezomib as it relies on FADD-caspase 8 mediated cell death signalling. Bortezomib and NPI-0052 were tested in combination and, given their different mechanism of action, they showed a synergistic effect both *in vitro*, in MM cell lines and patient MM cells and *in vivo*, in human plasmacytoma xenograft models.[8] Although NPI-0052 is orally active, in the phase I clinical trial that has been reported so far[9] the drug was administered as a weekly IV bolus. Sixteen patients with lymphoma or solid tumors were treated at doses ranging from 0.0125 to 0.112 mg/sqm for up to 7 cycles; a MTD was not reached and 4 patients showed stable disease for up to 4 months.

Carfilzomib (PR-171) is an epoxyketone related to epoxomycin, that is a natural product isolated from actinomyces.[10] This

compound inhibits the chymotrypsin-like activity of the 20s proteasome and induces apoptosis of MM cell lines and patient MM samples by activating both extrinsic and intrinsic pathways. Carfilzomib seems to possess enhanced anti MM activity as compared to bortezomib, it overcomes resistance to bortezomib or conventional chemotherapeutic agents, and acts synergistically with dexamethasone.

Two phase I clinical trials have been reported using Carfilzomib as a single agent in hematologcal malignancies; in both of them the drug was administered in consecutive days in order to obtain a more prolonged proteasome inhibition, based on preclinical testing. In the first trial[11] Carfilzomib was used as a daily IV push for 5 consecutive days on a 14-day cycle in patients with MM or non Hodgkin's lymphoma; the doses administered ranged from 1.2-20 mg/sqm; minimal effective dose (MED) was 11 mg/sqm. Five responses were obtained in 14 patients enrolled at ≥ MED and 4 further lymphoma patients showed disease stabilization for longer than 7 months. Dose limiting toxicities were febrile neutropenia and thrombocytopenia. In the second trial[12] Carfilzomib was administered as an IV push on days 1-2, 8-9, 15-16 of a 28-day cycle, at doses ranging from 1.2-27 mg/sqm; MED was 15 mg/sqm. An objective response or better was observed in 5 out 16 patients enrolled at ≥ MED; six additional patients showed stable disease; dose-limiting toxicities were thrombocytopenia and reversible increase in serum creatinine.

Other proteasome inhibitors have shown promising activity *in vitro* and in animal models. CEP-18770 is a reversible P2 threonine boronic acid derivative that, like bortezomib, inhibits chymotrypsin like activity of the proteasome.[13] The effects of this drug are similar to those displayed by bortezomib in terms of NFkB downmodulation and induction of apoptosis in MM cell lines and purified CD138 positive cells from patients; however, CEP-18770 is significantly less cytotoxic on normal bone marrow progenitors and more potent in inhibiting osteoclasts formation. Remarkable efficacy was also observed in MM xenografts. BSc2118 is a tripeptide compound that inhibits all three proteolytic activities of the 20s proteasome; this drug caused growth inhibition associated with downregulation of NFkB activity and cell cycle arrest in a mantle cell lymphoma model.[14] Clioquinol is a copper-binding halogenated 8-hydroxyquinoline that was used in the 1950's-1970's as an oral antiparasitic agent. In cell lines and primary AML samples this drug inhibited chymotrypsin-like activity of the 20s proteasome and caused reduction of cyclin D1 and cell cycle arrest.[15] This compound was able to induce cell death of acute myeloid leukaemia (AML) and MM cell lines and freshly isolated AML samples.

Proteasome inhibition is now considered a suitable selective antineoplastic target and bortezomib represents a major achievement for the treatment of MM; however, further studies are needed in order to identify drugs with a wider spectrum of action and a more prolonged activity against different proteolytic sites of the 20s proteasome.

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