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Book Review

Proteasome inhibitors in cancer therapy: death by indigestion

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Proteasome Inhibitors in Cancer Therapy. Cancer Drug Discovery and Development. By J Adams. Humana Press, Totowa, New Jersey: 2004. pp 312. ISBN: 1-58829-250-9

As Eugene Garfield said, while it is easy to recognize a good paper, it could be more difficult to recognize a bad paper. In fact, the results could be weak, but the conclusion could still be right, even though not fully supported by the data shown. Preliminary reports could also fall in this category. After all, it was not a Cell but a BBRC paper – only a little BBRC of three impact factor – describing a novel experimental model in which to study ATP-dependent proteolysis.[1] In a lysate from rabbit reticulocytes, where the proteolytic activity was not due to lysosomes (pH optimum of 7.8), they separated two fractions in a DEAE cellulose column, each one individually inactive, but after recombination of the two fractions ATP-dependent proteolysis was reconstituted. Immediately, Hershko and his young assistant, Ciechanover, went for a sabbatical to the Fox Chase Cancer Center in Philadelphia to work with Irwin Rose, and the three began unveiling the E1-E2-E3 ligase components of the Ubiquitin-Proteasome System (UPS),[2] see for example some recent reviews.[3,4] For this work, Ciechanover, Hershko, and Rose were granted the 2004 Nobel Prize. Five clinical trials based on UPS inhibition were in progress in the same year:[5] (i) Velcade-TM (dipeptide boronic acid, Bortezomid, PS-341, Millenium Pharmaceuticals Inc.) phase III, FDA approved for relapsed and refractory multiple myeloma and other solid cancers; it stabilizes cell-cycle and proapaoptotic proteins, inhibits antiapoptotic proteins, and affects tumor microenvironment; (ii) MLN519 (lactacystin derivative, PS-519, Millenium Pharmaceuticals Inc.) phase I for acute stroke and myocardial infections; it is a potent anti-inflammatory and neuroprotective compound; (iii) Epoxomicin-Eponemycin (Streptomyces epoxyketones) preclinical; it has cytotoxic effects in various tumor cells; (iv) NLVS (trileucine vinyl-sulfone) preclinical; it is an irreversible inhibitor of trypsin- and chemotrypsin-like proteasome activities; (v) Ritonavir (Peptidomimetic protease inhibitor; Abbott) phase II for AIDS and tumor patients; it is an HIV protease inhibitor, also inhibits chemotrypsin-like activity of proteasome. While the timeline of this story is outlined by Ciechanover himself,[6] the present book describes the most advanced ongoing clinical trials on UPS inhibition. It is of particular interest that this book review comes out in the same issue containing manuscripts from scientists that have originated in this field.[7–10]

For those of us engaged in basic research in the field of cancer and apoptosis, the hope that our work might, in some small way and at some future juncture, contribute to the clinical treatment of human cancers is a major motivator. *Proteasome Inhibitors in Cancer Therapy,* edited by Julian Adams, which is part of the 'Cancer Drug Discovery and Development' series from Humana Press, tells the happy tale of one drug's journey from concept, through development, to FDA approval, and application. The drug in question, developed by Millennium, is variously known as PS-341, bortezomib, and VELCADE™, and it is a potent inhibitor of the 26S proteasome.

This book is aimed as much at scientists interested in developing cancer drugs, as it is at those interested in the emerging field of proteasome regulation and inhibition. This is made clear from the first chapter, which is not an introduction to the function of the proteasome, but rather a primer on cancer drug development, under the heading 'Challenges in a Competitive Market'. This chapter gives a brief history of the market for cancer drugs, and lays out in stark numbers the economical considerations involved in the development of life-saving therapies. To scientists ensconced in the intellectual comfort of academia, this chapter may appear somewhat repugnant; it speaks with undisguised zeal about projections indicating 'a $17 billion oncology market by 2005' and '8 million cancer patients in the United States, Europe, and Japan by 2008,' the obvious implication being that cancer patients in poorer nations need not be considered for costly therapies. However, this chapter also dispenses 'a dose of reality,' pointing out that most initially promising cancer drugs never reach the market, costing drug companies billions of dollars in unrecoverable R&D expenses, and that when the FDA or EMEA does not approve a cancer drug, or requires additional trials, companies face additional costs of $10 to $30 million.

Having laid out the sizable challenges and rewards inherent in cancer drug development, the remainder of the book is dedicated to consideration of PS-341 as a case study in

successful drug design. The second chapter is penned by Alfred L. Goldberg, a pioneer in the field of proteasome research who cofounded MyoGenetics (which was subsequently absorbed by ProScript and later Millenium) with the original aim of developing proteasome inhibitors to slow the progression of muscle wasting diseases. He also recognized that inhibiting the proteasome was an excellent way to study its function, although he slyly refers to this as his 'hidden agenda' because 'venture capitalists, stockholders, and company managers are not solely motivated by their interest in advancing biological science'. In the end he got to have his cake and eat it too; MG132 (MG stands for MyoGenetics) remains the most used proteasome inhibitor in basic research, and, as the book notes again and again, PS-341 has been approved for treatment of multiple myeloma, and may be effective against other cancers. The original group of enzymologists (led by Ross Stein), chemists (led by Julian Adams), and cell biologists (led by Vito Palombella) at MyoGenetics led to the development of other UPS inhibitors, such as, for example, PS-519, now also in clinical trial.

In this and subsequent chapters, a variety of authors lay out the biological basis for proteasome inhibition in cancer therapy. The structure and function of the various components of the 26S proteasome are considered in detail, as are the modes of action of a large number of proteasome inhibitors, both synthetic peptide aldehyde (MG132, PSI, glyoxal, CEP1612, PEG), peptide amides and boronates reversible inhibitors (benzamide, α-ketoamide, bortezomide, Cbz-LLL-boronic acid), vinyl sulfones and epoxyketones irreversible inhibitors (H2N-LLLL-VS, Ac-PRLN-VS, Ac-YRLN-VS, YU101, YU102), and natural compounds (lactacystin, clasto-lactacystin β-lactone, epoxomicin, dihydroeponemycin, TMC-95A/B, gliotoxin, phepropeptin B, EGCG). Even though these compounds show different chemotrypsin-like, trypsin-like or peptidylglutamyl-peptide hydrolyzing proteasome inhibitory properties, their general *in vivo* activity can be significant, as for example, for lactacystin (4.0 μM), CEP1612 (1.0 μM), MG132 (0.4 μM), YU101 (0.25 μM), Cbz-LLL-boronic acid (0.04 μM), epoxomicin (0.03 μM), and bortezomide (0.02 μM). Interestingly, the green tea polyphenol component epigallocatechin-3-gallate (EGCG) shows anticancer properties, and NF-κB inhibition, probably due to its ability to inhibit the UPS.

A chapter by Julian Adams explains the role of the proteasome in the cell cycle, where it is intimately involved in cycle progression via degradation of the cyclins, as well as regulation of checkpoint proteins such as pRb and Survivin. Simona A. Williams & David J. McConkey explain the role of the proteasome and its inhibitors in apoptosis, including regulation of NF-κB, p53, and the caspases. In fact, both processes need a very tight regulation, where the half-life and degradation of specific proteins must be strictly regulated. Their UPS deregulation inevitably leads to apoptosis. Probably the most relevant survival regulator controlled by UPS is NF-κB, where the inhibitor IkB is degraded by the E3 ligase β-TrCP-targetted degradation. This section of the book closes with a discussion on the evaluation of PS-341 by the NCIs COMPARE algorithm, in which potential molecular targets are screened upon PS-341 treatment using a panel of 60 different tumor cell lines.

The book's third section, titled 'Rationale for Proteasome Inhibitors in Cancer,' begins with some general biology and progresses toward clinical applications of PS-341. Initial chapters consider the role of the proteasome in tumor cells, and the biological basis for the striking finding that proteasome inhibition slows the growth of tumor cells. It seems that, like oncogenesis, proteasome inhibitor-induced apoptosis is the result of several interrelated mechanisms. These may include cell cycle blockage due to inhibited cyclin clearance, caspase induction, IAP stability, p53 accumulation, and NF-κB inhibition. A major role seems to be exerted by NF-κB degradation (see above), which controls several survival signals (IL-6, IL-8, TNF-α, TNF-β, VCAM, ICAM, ELAM, IAP, several oncogenes), very important in cancer cell survival. The fact that proteasome inhibition can induce growth arrest via multiple pathways may explain the effectiveness of this type of therapy in checking runaway division resulting from many kinds of lesions in the cell cycle machinery.

Later chapters consider the potential effectiveness of PS-341 in combination with a variety of established radiation and chemical-based therapies, including a detailed analysis of the biochemical bases for these combinations. Proteasome inhibition is found to have a sensitizing effect on tumor cells, leading to positive outcomes at lower doses in both radiation therapies and in response to compounds such as doxorubicin, docetaxel, cisplatin, and TRAIL. *In vivo* experimental models of tumor growth in mice and rats, with either intravenous or intratumoral administration, produce promising safety and efficacy. Most important, UPS inhibition reverted drug resistance of cancer cell lines, suggesting an intriguing interference with the stability of drug resistance-related proteins such as the MDR pumps P-gp, Mrp 3, Mrp 5, or topoisomerase II. The UPS inhibitors *N*-acetyl-leucyl-leucyl-norleucinal (ALLnL) and lactacystin potentiate cisplatin-induced apoptosis and revert their drug resistance by inhibiting the NER-dependent repair of cisplatin-DNA adducts. This occurs via two distinct mechanisms. First, by proteasome inhibitor-caused depletion of ubiquitinated histone H2A in nucleosomes, which promotes chromatin condensation and possibly interferes with the function of DNA damage recognition and repair enzymes. Second, proteosome inhibitors diminish the excision repair cross-complementation group 1 (ERCC-1) response to cisplatin, perhaps secondary to changes in chromatin structure that interfere with the transcription of the ERCC-1 gene. Interestingly, ubiquitin was first identified as the mono-ubiquitinated form of histone H2A, named 'protein A24', an odd protein with two N-terminus and one C-terminus.[11] Surprisingly, the function of mono-ubiquitinated histone H2A is still unknown, but it appears to have nothing to do with protein degradation. Nevertheless, more work is needed to fully understand the mechanistic basis of the relationship between proteasome and sensitivity to cisplatin and other chemotherapic drugs.

Ritonavir (ABT-538, Abbott), see above, is a UPS inhibitor that shows a specificity for the protease encoded by HIV-1, able to inhibit viral replication at 20–130 nM. In fact, several UPS inhibitors retarded budding, maturation, and infectivity of HIV.

The book's final section is dedicated PS-341's clinical trials. While of limited interest from a purely biochemical standpoint,

these chapters enumerate the many considerations and challenges inherent in shepherding a drug through this process, and as such could prove valuable to drug developers. Indeed, as mentioned previously, this book is directed as much to those interested in drug development as to scientists working on the proteasome. In some cases, descriptions of the biological underpinnings of key processes are somewhat cursory or rushed, and scientists expecting an extended review of proteasome function and inhibition could be a bit disappointed; sections of the book read like an extremely detailed advertisement for PS-341. Of course, many of the authors were involved in PS-341's development, and as such they can be forgiven a certain preoccupation with what appears to be an excellent and interesting compound. Furthermore, as the first proteasome inhibitor to be granted FDA approval, it is only natural that PS-341 should figure heavily in any discourse on proteasome inhibitor-based therapy.

The three initial phase I trials (MDACC 98–194 at M.D. Anderson Houston; MSKCC 98–104 at Memorial Sloan-Kettering; UNC/MSKCC 98-034-031 at Chapel Hill North Carolina) indicated that 1.0–1.5 mg/m^2 bortezemid twice weekly produced a 40% UPS I inhibition, with a recovery at 72 h. Toxicity showed peripheral sensory neuropathy and diarrhea, but notably no hematological events, with rare thrombocytopenia, febrile neutropenia, hepatic-, renal-, cardiotoxicity. UPS inhibitors were particularly effective in combination-treatment regimen, where the addition of UPS inhibitors to chemotherapy (e.g. gemcitabine, doxocycline) did not change toxicity. Probably, a significant effect of bortezemid seems to be related to the constitutive hyperactivity of NF-κB of cancer cells, which is specifically inhibited by UPS inhibitors. A further effect useful for cancer therapy is the ability of bortezemid to inhibit angiogenesis.

While in cancer cells bortezemid induces apoptosis, in normal cells UPS inhibitors are either ineffective or protect from apoptosis, such as in thymocytes[12] and neurons.[13] Therefore, the proapoptotic effects could not be generalized. It is still unclear what regulates the cellular response to UPS inhibition. With regard to this, the COMPARE analysis was quite unsatisfactory (see chapter 8). Several mechanisms could be proposed: (i) tumor cells may have an aberrant balance of survival signals (e.g. NF-κB, NGF, Bcl-2); (ii) they could contain lower levels of deubiquitinating enzymes, thus causing the accumulation of toxic aggregates; (iii) tumor cells might possess an altered chaperone network; (iv) the ER stress response might be different in tumor *versus* normal cells; (v) negative feedback mechanisms acting on protein synthesis could be altered in tumor cells. This suggests that possibly the best selectivity should not be searched at the proteasome level, but rather upstream, at the E3 ligase level. To date, no E3-specific inhibitors have been developed, but the race has just started.

A general fault of this book is that chapters are often quite repetitive. For example, chapters 3, 4, and 5 contain paragraphs about the same UPS inhibitors. Moreover, the two chapters focused on virus/proteasome connection, although very interesting and well written, have several redundant parts. Finally, introductory paragraphs on the NF-κB pathway are present in at least four chapters (7, 9, 11, 14). This is, however, a problem characterizing many scientific books, where chapters seem quite disconnected from each other and comprehensive introductory sections are missing. Should you read this book or do you have alternatives? If your flight is too short, and consequently you do not have the time to read 312 pages, carry 200 g extra luggage (and you prefer your iPod), and prefer a predigested manuscript, then the same Editor, Julian Adams, has published a very comprehensive, nice, and detailed review that summarizes the entire book.[14]

What can we conclude at this stage? The results of the phase I and II trials using the UPS inhibitor bortozemid (Velcade-TM) showed excellent safety (some gastro-intestinal toxicity with periferal neuropathy; no additive toxicities in combination with gemcitabicine, irinotecan, doxorubicin) with good efficacy (10% complete response and 18% partial response in 202 myeloma patients, 91% of which refractory to therapy; overcoming of chemoresistance *in vitro* and in patients). The phase III trial started in 2003. This is only 20 years after the discovery of the UPS by Aaron Ciechanover, Avram Hershko, Irwin Rose (Nobel Prize, December 2004). These new inhibitors are a new class of anticancer agents, acting on the UPS, undoubtedly providing valuable tools for therapy. However, to achieve more specificity, and thus increase the safety and efficacy of therapeutic agents targeting the UPS, the intervention of upstream proteasome degradation is desirable, that is ubiquitination by E3 ligase. This will be the object of the forthcoming research. One can only hope that, for the good of us all, these and other similar therapeutic agents live up to their considerable promise.

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