PREFACE

The proteasome field has exploded in the years since the proteasome's discovery in the early 1990s. The proteasome is a highly conserved multicatalytic protease that is responsible for cellular protein turnover, and by definition governs critical processes in cell biology. This field is no less complex and exciting than the more trodden path of transcription and protein synthesis. The unique biochemistry of the proteasome as one of nature's most fascinating proteases has allowed chemists to develop synthetic inhibitors of this most intriguing enzyme. While chemists applied their skills to develop mechanism-based inhibitors, it was also revealed that Mother Nature had evolved her own inhibitors, natural products, secondary metabolites, all with origins in bacteria.

Although all these investigations represent an enzymologist's dreamscape for academic investigation, the development of "tool drugs" to inhibit the proteasome has allowed an even more impressive number of studies in cell biology to interrogate the function of the ubiquitin proteasome pathway in numerous cell lines. Such research has allowed the determination of the function, temporal presence of short-lived proteins, antigen presentation, cell cycle regulation, transcriptional activation, cell adhesion, and apoptosis, to name a few processes.

One common feature was inevitably revealed. Inhibition of the proteasome in cultured cells, mostly of tumor origin, produced profound stabilization of hundreds, if not thousands, of proteins, ultimately turning on the programmed cell death machinery, at concentrations that directly correlated to the intrinsic inhibition constant of the proteasome. Such observations begged further investigation of proteasome inhibition in the treatment of human cancers. At first consideration, it would appear that it should be "illegal" to inhibit the proteasome and that a reasonable therapeutic index could simply not be achieved. Indeed, there was much reasonable skepticism in the scientific community that proteasome inhibitors could be safely used in animal studies, much less to treat human patients with cancer. No responsible scientist could fault such a negative view of the use of proteasome inhibitors in vivo. However, as we will see revealed in the chapters of *Proteasome Inhibitors in Cancer Therapy*, there is indeed a well-developed body of empirical evidence that the proteasome is a viable target to treat human diseases. *Proteasome Inhibitors in Cancer Therapy* focuses on the role of the proteasome inhibitors in cancer, for that is the most advanced body of knowledge to date, but as we shall see there are hints and data that the proteasome can also treat vascular diseases, viral infections, and possibly other maladies. Whether any of these investigations eventually lead to a practical treatment in human patients remains to be seen. At the time of this writing, the data seem to be mounting that the treatment of hematological diseases, especially multiple myeloma, would indicate a potential for the introduction of a novel important contribution to these deadly cancers.

The compelling reason for editing and contributing to this compilation of scientific studies of the proteasome arose from our desire to assemble a set of chapters describing the discovery of the basic enzymology and cell biology, combined with the creativity of

medicinal chemistry, to take a field of limited academic interest and show that in less than a decade drug candidates are testing the practical utility of proteasome inhibition in cancer. The story flies in the face of conventional wisdom. Moreover, some of the chemical matter embedded in these inhibitors also represents a break from conventional drug substances. Mechanism-based inhibitors are rare in the pharmacopoeia, but the proteasome begs to be inhibited by such odd substances as boronic acid peptides, β-lactone natural products, peptide epoxides, and complex depsipeptide structures. The boronic acid PS-341 (bortezomib) represents the most advanced of these agents and a following Phase III randomized clinical trial in an international multicenter, has recently been approved by the FDA for use in multiple myeloma. This molecule features prominently in many of the chapters as being the first, and ground breaking, drug, but I expect that it is but the beginning of many exciting therapeutics in the field.

Proteasome Inhibitors in Cancer Therapy is divided into four parts: The first part addresses the broader issues of the complexities and challenges of drug development for new cancer agents, in an ever competitive market, with changing standards of care and treatment combinations. Greene provides a most insightful analysis of the world of oncology addressing very practical and economic considerations.

The next chapters address the basic biochemistry and early discoveries in cell biology. Alfred Goldberg, one of the early pioneers, and founder of Myogenics (later becoming ProScript) teaches us in his chapter the history and mechanism of the proteasome. Subsequent chapters address natural product and synthetic inhibitors, which enabled the brilliant work of Robert Huber and his colleagues to define the three-dimensional structure of this awesome proteolytic machine. Subsequent contributions reveal the role of the proteasome in the cell cycle and apoptosis. The National Cancer Institute played a formidable public service in the comprehensive assessment of the inhibition of the proteasome in their 60-tumor cell panel and used sophisticated informatics to correlate proteasome inhibition and inhibition of cell growth and apoptosis. One may argue that the proteasome inhibition is a sledge hammer approach to blocking cellular protein turnover. Read and Brownell teach us that using proteasome inhibitors as tools, we may reveal more important medicinal targets in the ubiquitination machinery to target a subset of proteins that govern very restricted functions in cells.

The third part of the book addresses the very empirical and practical development of rationales to test proteasome inhibitors in cancer models. A major contribution, originally made by Maniatis, Goldberg, and colleagues at Harvard, and subsequently extended by Cusack and Baldwin and others, was elucidating of the role of the proteasome in NF-κB activation. DNA damage leads to a profound activation of the transcription factor that can be abrogated by proteasome inhibitors. This part of the book documents the generality of combining conventional chemotherapy and radiation with proteasome inhibition, notably PS-341. Schubert has extended the possibility that proteasome inhibitors may provide a new method of inhibiting viral maturation and budding by targeting the supporting cellular structures that assist retrovirus release from infected cells. Another remarkable contribution from Groettrup and colleagues reveals that the HIV protease inhibitor, ritonavir, is itself a proteasome inhibitor and may be part of its proven efficacy in the treatment of AIDS. Though ProScript, now a part of Millennium Pharmaceuticals, was the first to introduce a proteasome inhibitor into human trials, we note that other pharmaceutical companies have been active in the field and this is documented by García-Echeverría, from Novartis.

Part IV of this book represents a work in progress, documenting the development of bortezomib (VELCADE™) in clinical trials. The preclinical development allowed for the selection of doses and schedules that could be translated to human patients. Perhaps the most important element was the use of a pharmacodynamic assay to monitor proteasome inhibition in the blood to ensure that partial and temporal inhibition was maintained in a manner that could be tolerated by patients. The Phase I investigations are a joint effort of Millennium-sponsored trials together with the NCI extramural sites. The important contribution by Anderson, considered one of the leading authorities in multiple myeloma research and treatment, describes the activity of bortezomib in a multicentered Phase II clinical trial in patients with relapsed and refractory myeloma. Proteasome inhibition in myeloma and other diseases is also being pioneered using modern pharmacogenomic tools to assess which patients will be predicted to respond to therapy. Ross and colleagues describe the potential for such techniques to accompany proteasome inhibitor therapy.

Proteasome inhibition has certainly consumed my life for almost a decade and I feel privileged and fortunate to have been part of the emergence of this field, and participate in what I believe to be a fertile arena for many future discoveries, which my instincts tell me will provide much relief of suffering and extend quality life to patients afflicted with cancer and other debilitating diseases. I hope *Proteasome Inhibitors in Cancer Therapy* can provide some useful teachings for students, professors, and industry researchers alike.

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