PREFACE

Protein kinases function as components of signal transduction pathways, playing a central role in diverse biological processes, such as control of cell growth, metabolism, differentiation, and apoptosis. The development of selective protein kinase inhibitors that can block or modulate diseases with abnormalities in these signaling pathways is considered a promising approach for drug development. The function of many protein kinases is deregulated in human cancers. Deregulation, whether as a result of deletion, mutation, or amplification, is manifested as aberrant activation, prime examples of which are kinases including Bcr-Abl, EGFR family members, Flt-3, Met, etc., as well as kinases involved in the neovascularization of tumors like KDR. A decade ago, these protein kinases were considered prime targets for the development of selective inhibitors. Currently, over 20 different kinases—the majority being receptor protein tyrosine kinases (RPTKs)—are being considered as potential therapeutic targets in oncology.

Although the success of agents such as Glivec® (Imatinib mesylate, Glivec/Gleevec®) and Iressa™ (Gefitinib) has provided a proof of concept that such agents can be therapeutically effective and retain an acceptable safety profile, the clinical experience with other tyrosine kinase inhibitors is still limited.

A comprehensive overview of the drug discovery processes aimed at generating inhibitors for the treatment of malignancies believed to be dependent on the gain of function of protein tyrosine kinases (PTKs) has to contain a summary of those drug discovery programs that have devoted their efforts to generating low molecular-weight (LMW) inhibitors directed against either the adenosine triphosphatase (ATP)-binding site (summarized in Chapter 1) or the Src homology 2 (SH2) domain, an important noncatalytic module that recognizes a short phosphotyrosine-containing sequence in other proteins. A review on the advances made targeting this critical SH2-binding event, which would result in the inactivation of undesirable signal transduction networks, is found in Chapter 2.

Epistatically, PTKs are located either upstream and/or downstream of tumor suppressor genes or oncogenes and have been demonstrated to play central roles in apoptosis, proliferation, invasion, and differentiation. The signal transduction pathways of PTKs, in particular receptor PTKs, is intimately linked to the phosphoinositide 3-kinase (PI3-K) pathway as activation of cells by a wide variety of stimuli leads to rapid changes in 3-phosphorylated inositol lipids through the action of a family of enzymes known as PI3-Ks. The dissection

of PI3-K signaling pathways has been greatly aided by genetic approaches and by the availability of two pharmacological tools, wortmannin and LY294002. In Chapter 3, a comprehensive summary is given to explain why the PI3-Ks represent a reasonable target for pharmaceutical intervention. All the reasoning for the activation of PI3-K as target is central to the coordinated control of multiple cell-signaling pathways leading to cell growth, cell proliferation, cell survival, and cell migration.

Aberrant activation of tyrosine kinases, owing to mutation or overexpression, is sufficient for them to become transforming in cellular and animal models. The majority of targets are RPTKs. Deregulating mutations of over half of the known RPTKs have been associated with different human malignancies. To illustrate the rationale and the progress made towards generating "selective" LMW kinase inhibitors, a few selected examples have been chosen that include the targets of Glivec (platelet-derived growth factor receptor, Kit, Bcr-Abl), FLT-3, JAKs, as well as Src. A special chapter has also been devoted to the normal function, role in disease, and application of platelet-derived growth factor antagonists. All of these efforts illustrate the tremendous biological complexity that is encountered by targeting these kinases and render a conclusion about the actual level of understanding of the molecular epidemiology and pathophysiology, as well as disease relevance of these kinases. In particular, the success story of Glivec has taught the academic, as well as pharmaceutical fields, some lessons regarding the inhibition of these kinases from the points of view of therapeutics and biology (Chapters 4–8).

A successful development of protein kinase inhibitors is based primarily on solid epidemiology allowing the identification and validation of the target along with the knowledge of the structure of the kinase. The structural understanding of protein kinases has significantly progressed as structures of kinases both in phosphorylated or nonphosphorylated forms, active or inactive states, unliganded or complexed to substrate analogs or inhibitors, and with only the catalytic domain present or in a multidomain construct including SH3 and SH2 domains have become available. All of this knowledge is being used for structure-based design and has been summarized in Chapter 9.

Robust predictive preclinical in vitro and, in particular, in vivo screening model systems that allow rapid optimization of lead compounds are key to a successful drug discovery effort as they are crucial for determining the safety of kinase inhibitors. Animal models as used in cancer can be divided conveniently into models designed to understand the natural history of cancer and models that are useful for the testing, selection, and profiling of new anticancer treatment modalities. The advantages and disadvantages of in vivo preclinical models for testing protein kinase inhibitors with antitumor activity have been summarized in Chapter 10.

Finally, one of the most important steps in the drug discovery process leading to kinase inhibitors is to determine "on-target" vs "off-target" effects by demonstrating that the protein kinase inhibitor downregulates the function of the target in vitro and in vivo with all the expected consequences (downregulation of given pathway[s] and growth arrest). Therefore, phosphoprofiling or phosphoproteomics that include the large-scale determination of protein phosphorylation in cells and tissue is one approach that can be used to characterize biological states, including therapeutic responses to provide a comprehensive picture of cellular states. The utility of these methods in drug discovery and development is discussed in Chapter 11.

Understanding the role of a potential target in cancer development and progression is as relevant as the efficient optimization of an inhibitory compound's potency, toxicity, and pharmacokinetic profile. To be a valid target, a kinase should play a fundamental role in the pathogenesis of a disease and the rationally designed LMW compounds, which are almost exclusively directed against the ATP-binding site of the kinase, should be able to revert the effects of the disease-causing kinase in preclinical models, and should be translatable to clinical settings.

Doriano Fabbro, ***PhD***
Frank McCormick, ***MD, PhD***