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Review

Therapeutic Protein Kinase Inhibitors

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Abstract. Protein kinase inhibitors represent an important and still emerging class of targeted therapeutic agents. Drug discovery and development strategies have explored numerous approaches to target the inhibition of protein kinase signaling. This review will highlight some of the strategies that have led to the successful clinical development of therapeutic protein kinase inhibitors, particularly as anticancer drugs. Some notable advances have been made in the development of novel protein and oligonucleotide-based biologics that target growth factor or receptor tyrosine kinases. Also, advances have been made in the rational design of small-molecule inhibitors that target unique kinase conformational forms and binding sites, and have specific kinase selectivity profiles. A review will also be given of some of the potential clinical toxicities and adverse side-effects associated with these kinase-targeted drugs. Therapeutic protein kinase inhibitors have been highly beneficial to cancer patients and offer the promise of future therapies for other diseases as well.

Keywords. Protein kinase, kinase inhibitor, kinase signaling, drug discovery.

Introduction

The reversible phosphorylation of proteins represents a major post-translational signaling mechanism and regulatory pathway that controls a diverse set of cellular processes. The phosphorylation of proteins is catalyzed by protein kinases, representing a large family of ATP-dependent phosphotransferases from as many as 518 putative kinase genes that make up the human kinome [1]. Protein kinases catalyze the reversible hydroxyl-phosphorylation of Tyr, Ser, or Thr residues of protein substrates. Often the protein kinase itself is the substrate for an upstream kinase or undergoes autophosphorylation as part of a cascade of protein kinase signaling within the cell.

The mapping and elucidation of protein kinase signal transduction pathways has been an extensive cell biology area of research. Some representative protein kinase signaling pathways within cells include growth factor signaling and stress-activated signaling responses (Figure 1). Such pathways are highly interconnected and complex (much more so than the linear protein kinase cascades represented in Figure 1) and regulate numerous cellular functions such as gene transcription, cell growth, proliferation, and differentiation.

Indeed, the history of protein kinase research is immensely rich with many notable biological discoveries. This includes pioneering biological research leading to several Nobel Prize awards in Medicine such as the seminal studies by E. H. Fischer and E. G. Krebs on protein phosphorylation and regulation of biological processes [2–4]. In 1989, J. Michael Bishop and Harold Varmus were recognized for another important discovery that some protein kinases can act as oncogenes [5]. Aberrant protein kinase activity can disrupt the normal control of cellular phosphorylation

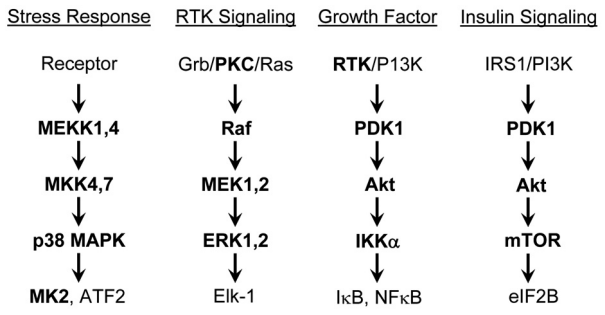

Figure 1. Examples of protein kinase signaling cascades. Four representative cellular signaling pathways are shown that incorporate a stimulus (e.g., receptor activation by binding of a growth factor), initiation of a series of kinase reactions (e.g., pyruvate dehydrogenase kinase, isoenzyme 1 (PDK1) phosphorylation of protein kinase B (Akt)), and activation of transcription factors (e.g., nuclear factor κB (NFκB)). Kinases are shown in bold font.

signaling pathways and lead to tumor formation. Protein kinases are also involved in molecular signaling pathways of the cell cycle, regulating eukaryotic cell growth (interphase), replication (mitosis), and programmed cell death (apoptosis). In 2001, Paul Nurse and Timothy Hunt received the Nobel award for describing the role of cyclins and cyclin-dependent kinases that regulate the sequential phases of the cell cycle [6, 7]. Leland Hartwell was a co-recipient, recognized for developing the checkpoint concept for cell cycle arrest that allows for DNA repair through a checkpoint kinase signaling cascade [8, 9].

Given the critical role that protein kinases have in modulating cellular functions such as tumorigenesis, this class of enzymes has been targeted for the discovery and design of biologics and small-molecule inhibitors as potential therapeutic agents. Over the past decade, over a hundred different protein kinase inhibitors have entered clinical trials.

While the development of therapeutic inhibitors of protein kinases has found most success in anti-cancer therapy, this class of targeted inhibitors has the potential for modulating diverse diseases where protein kinase activity plays an etiological or pathogenic role. Therapeutic protein kinase inhibitors are in clinical development for diseases such as rheumatoid arthritis, cardiovascular disease, diabetes, and diabetic complications. This review provides an overview of the drug discovery strategies, innovations, and challenges that have led to the successful design and development of therapeutic biologics and small-molecule protein kinase inhibitors. An emphasis is placed on the application of structure-based drug design, kinase selectivity profiling, and a discussion of the drug safety and toxicity issues related to this class of pharmaceutical agents. The focus is on those therapeutic protein kinase inhibitors that are currently in late-stage clinical studies or have successfully progressed through clinical trials.

Biotherapeutic protein kinase inhibitors targeting growth factor signaling and angiogenesis

Receptor tyrosine kinases (RTK) are cell-surface receptors with an extracellular domain that selectively binds and is activated by various growth factors, such as epidermal growth factor (EGF), insulin-like growth factor (IGF), or vascular endothelial growth factor (VEGF). Upon binding of these growth factor ligands, the RTK dimerizes and activates the intracellular protein kinase domain, resulting in the further activation of signal transduction pathways (see Figure 1). Numerous therapeutic biologics have been successfully developed that inhibit RTK signaling and modulate cellular functions such as aberrant cell growth (tumorigenesis) and angiogenesis. Various types of biotherapeutic drugs have been pursued and include therapeutic monoclonal antibodies, vaccines, and other novel oligonucleotide-based agents.

Therapeutic monoclonal antibodies have been developed that specifically bind to distinct growth factors or RTKs such as Avastin® (bevacizumab, Genentech/Roche) that binds VEGF [10, 11] and Erbitux® (cetuximab, ImClone) that blocks the EGF receptor tyrosine kinase (EGFR) [12, 13]. In this way, these biotherapeutic antibodies can prevent the growth factor/RTK interaction and thus inhibit the RTK-dependent signaling pathway [14–16]. Other therapeutic antibodies that block protein kinase signaling are also in clinical development or have already been approved for cancer immunotherapy. For breast cancer, the monoclonal antibody Herceptin® (trastuzumab) was developed by Genentech against the RTK extracellular domain of ErbB2 or HER2 [17–20]. Pertuzumab (Omnitarg, Genentech) is a novel therapeutic antibody in clinical studies for colon cancer that acts by blocking the dimerization of the HER2 and HER3 RTKs [21, 22]. The two anti-VEGF antibodies, Avastin and Lucentis® (ranibizumab, Genentech/Novartis) are effective in blocking the angiogenic effect of VEGF in neovascularization of tumors [23, 24] or associated with wet age-related macular degeneration (AMD), a leading cause of blindness [25, 26]. Biotherapeutic antibodies for EGFR include the chimeric monoclonal antibody Erbitux, and the humanized monoclonal antibodies Vectibix® (panitumumab, Amgen) [27, 28] and the clinical candidate, nimotuzumab (YM Biosciences) [29, 30]. Other targeted immunotherapeutics in late stage clinical development include tanezumab (RN624, Pfizer), a humanized monoclonal antibody

against nerve growth factor in patients with osteoarthritic pain [31, 32], and several potential anticancer antibodies against IGF-1R [33, 34]: CP-751,871 (Pfizer) [35], IMC-A12 (ImClone Systems) [36], MK-0646 (h7C10, Merck) [37], AMG-479 (Amgen) [38], and AVE1642 (ImmunoGen/Sanofi-aventis) [34].

Immunotherapeutic vaccines are also under investigation using growth factors, RTKs, or other protein kinase-derived peptides as antigens. Some promising investigational vaccines are in clinical studies (alone or in combination), particularly to boost the immune response against tumor cells. A number of HER2 peptide-based vaccines are in clinical testing for treatment of breast cancer such as a dHER2 vaccine (GSK) [39] and a HER2 intracellular domain peptide-derived vaccine (Univ. Washington) [40]. CimaVax EGF (Bioven) is a therapeutic vaccine recently approved for use in Cuba and in clinical trials elsewhere in patients with lung and other cancers [41]. CDX-110 (AVANT Immunotherapy/Pfizer) is an EGFRvIII-targeted vaccine that just completed Phase II clinical trial in glioblastoma multiforme patients [42–44]. A vascular endothelial growth factor receptor tyrosine kinase (VEGFR)-peptide vaccine (Tokyo University) is also being tested in various cancer trials and has already shown that it can induce an effective tumor specific cytotoxic T lymphocyte response [45]. The oncogenic BCR-ABL kinase has also been investigated as a potential antigen for development of a vaccine for the treatment of chronic myelogenous leukemia. Early immunological studies with various peptide-based and cell-based antigens using BCR-ABL or BCR-ABL-products have shown some initial immune responses, but they seem to be more effective when coupled with other chemotherapeutic agents [46, 47].

Similar to the anti-angiogenesis approach of targeting VEGF by anti-VEGF antibodies, other novel biologic therapies have been developed to block VEGF/VEGFR receptor kinase signal transduction that is involved in angiogenesis and blood vessel formation. Macugen® (pegaptanib, OSI/Pfizer) is a pegylated aptamer, a short-stranded oligonucleotide, which potently binds VEGF with high specificity. Macugen is effective in treating the neovascularization and microvascular leakage associated with AMD [48, 49].

Small interfering RNAs (siRNA) are double-stranded RNA molecules that act to silence gene expression and thereby reduce expression of a protein target and its functional activity. Several therapeutic siRNAs are also in clinical development for wet AMD that target either VEGF or VEGFR, such as bevasiranib (OPKO Health) [50] and Sirna-027 (Sirna Therapeutics/Merck) [51]. Another strategy is the use of soluble receptor fragments that mimic the extracellular domain of the RTK and effectively compete with the binding of growth factors to its receptor. Regeneron has produced fusion proteins, called "Traps" that combine the high-affinity receptor domains with an antibody Fc portion to create stable molecules that potently bind specific proteins, such as VEGF [52, 53]. Aflibercept (Regeneron/Sanofi-aventis) is a VEGF Trap in cancer trials and VEGF Trap-Eye (Regeneron/Bayer Healthcare) is in clinical testing for the treatment of AMD.

Antisense technology is another promising therapeutic strategy that seeks to decrease protein expression by use of short, complementary oligonucleotide, single-stranded DNA molecules that specifically bind to and interfere with the normal translation of messenger RNA. While Vitravene® (Isis Pharmaceuticals) is the only antisense-based drug approved to date, several antisense compounds for blocking protein kinase signaling are in various stages of clinical development. Affinitak™ (aprinocarsen, Isis/Lilly), for example, is an antisense oligonucleotide against protein kinase C-alpha that was in clinical trials in non-small cell lung cancer patients [54, 55]. AP 12009 (Antisense Pharma), is an antisense drug against the transforming growth factor-beta in late stage trials in high-grade glioma patients [56, 57]. Also, iCo-007 (ISIS 13650, ISIS/iCo therapeutics) is an antisense inhibitor of c-Raf mRNA in early-stage clinical testing in patients with diabetic macular edema [58].

While biologic therapies have shown efficacy in treating some cancers and other diseases, there are some limitations to their effectiveness. Some technical limitations for biotherapeutics concern their chemical stability or their inability to penetrate tissues and reach their intracellular targets. Some innovative drug formulation technologies have improved the stability and cellular targeting of oligonucleotide-based therapeutics such as encapsulation within liposomes, attachment to polymers, or through pegylation [59, 60]. Since multiple oncogenic pathways are often involved in tumor progression, the high selectivity of biotherapeutics for specific molecular targets has somewhat limited their effectiveness as single-agent anti-cancer therapies.

Small-molecule therapeutic inhibitors of protein kinases

A number of natural products from plant or microbial sources with anticancer activity have been shown to inhibit protein kinases involved in cellular proliferation, replication, and apoptosis. Examples of natural products that are potent inhibitors of protein kinases include the alkaloid staurosporine [61], the flavonoid

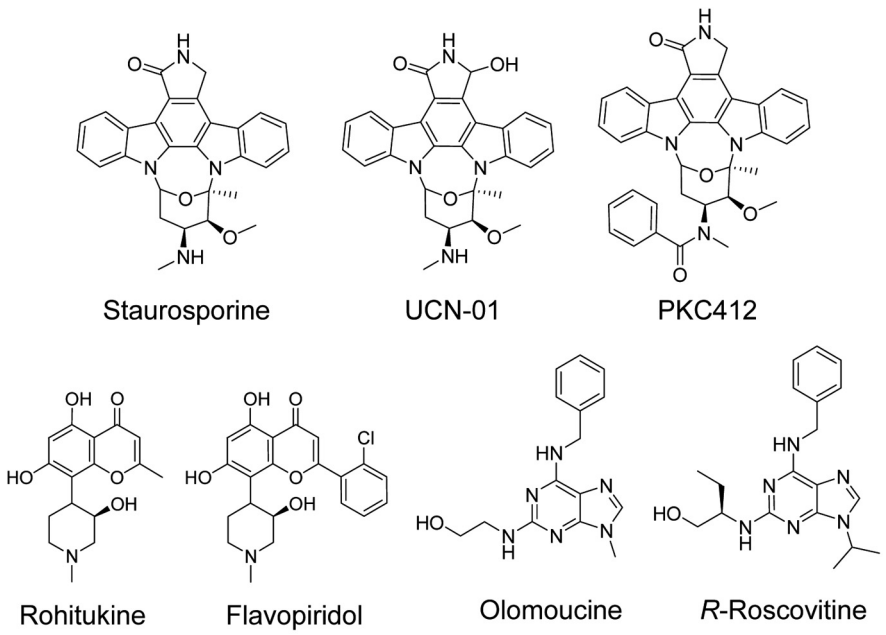

Figure 2. Natural product-based protein kinase inhibitors.

rohitukine [62], and the purine olomoucine [63, 64]. Many therapeutic inhibitors of protein kinases are structurally based on natural products such as these and are presently in clinical testing (Figure 2). UCN-01 (7-hydroxystaurosporine, Kyowa Hakko) and PKC412 (midostaurin, *N*-benzoyl-staurosporine, Novartis) are staurosporine-derived, kinase inhibitors in various oncological clinical studies [65, 66]. Flavopiridol (alvocidib, Sanofi-aventis) [67, 68] and *R*-roscovitine (seliciclib, Cyclacel) [69, 70] are inhibitors of cyclin-dependent kinases in cancer trials and are structurally related to rohitukine and olomoucine respectively.

Small-molecule inhibitors of protein kinases typically prevent either autophosphorylation of the kinase or subsequent phosphorylation of other protein substrates. One aspect that contributes to the high druggability of protein kinases is that they all have well formed binding sites for adenosine triphosphate (ATP), the phospho-donor for the phosphorylation of protein substrates. From the early days of protein kinase drug discovery, small-molecule inhibitor approaches that target the ATP site have come under criticism regarding the ability to achieve cellular potency and target selectivity. One argument was that an ATP site-directed inhibitor would not be able to effectively compete against the high intracellular ATP concentration in order to potently block protein kinase activity and signal transduction. This was based on the fact that most protein kinases have affinities for ATP in the 10–300 micromolar range while the intracellular concentration of ATP is much greater, around 1–2 millimolar. A second common skepticism was that the overall sequence homology for the amino acid residues within the kinase ATP binding sites would not allow for the development of a selective, ATP-competitive inhibitor.

The development of Gleevec® (Glivec® in EU, imatinib, Novartis), the first approved small-molecule protein kinase inhibitor [71], and numerous other drug and clinical candidates has alleviated much of this early skepticism. To date, eight small-molecule therapeutic protein kinase inhibitors have been FDA-approved within the US (Figure 3). All eight are indicated for the treatment of oncological diseases. These compounds can be generally classified depending on the protein kinase that they target: (1) the BCR-ABL fusion protein kinase; (2) the human epidermal growth factor receptor tyrosine kinases, HER1/EGFR1 or HER2/ErbB-2; or (3) the vascular endothelial growth factor receptor tyrosine kinase (VEGFR). Gleevec [72, 73], Sprycel® (dasatinib, BMS) [74, 75], and Tasigna® (nilotinib, Novartis) [76, 77] are inhibitors of BCR-ABL fusion protein kinase, an oncogene for chronic myeloid leukemia. Iressa® (gefitinib, AstraZeneca) [78], Tarceva® (erlotinib, OSI/Genentech) [79], and Tykerb® (lapatinib, GSK) [80] are inhibitors of EGFR family members and block the tumorigenic effects of these RTKs. Sutent® (sunitinib, Pfizer) [81–84] and Nexavar® (sorafenib, Bayer/Onyx) [85] inhibit VEGFR and other protein kinases involved in tumor angiogenesis. Some of these compounds also inhibit other kinases in addition to those described above. For example, imatinib (BCR-ABL inhibitor) [72, 73] and sunitinib (VEGFR inhibitor) [82, 83] also inhibit KIT (c-kit

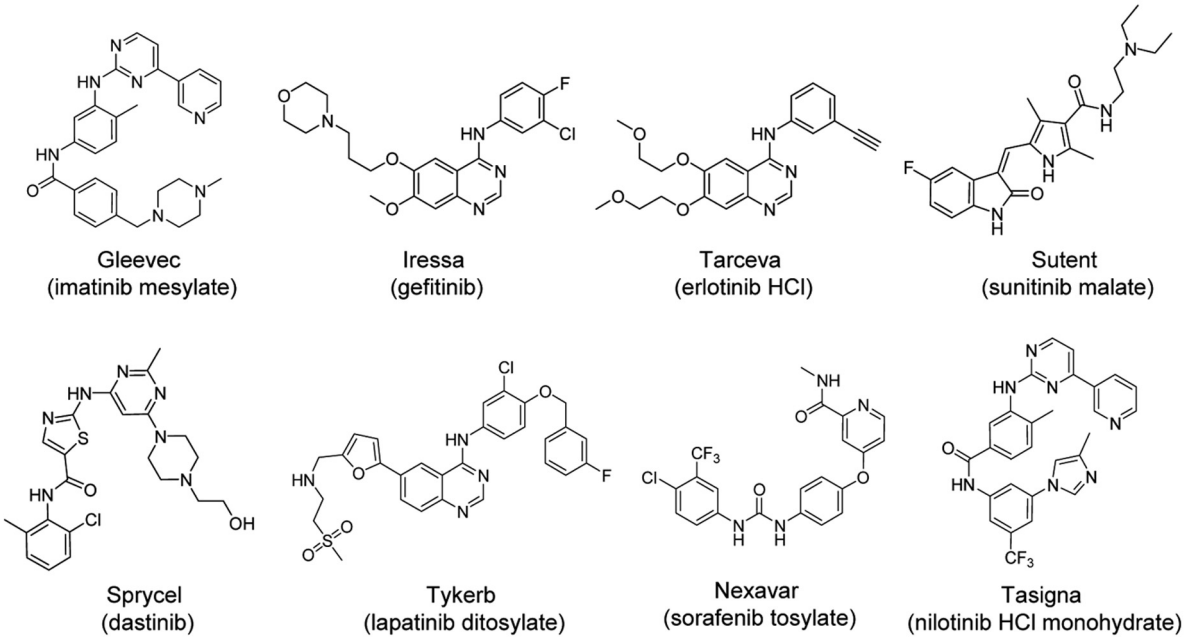

Figure 3. US FDA-approved, small-molecule therapeutic protein kinase inhibitors.

receptor, also known as cytokine stem cell factor receptor tyrosine kinase) and the platelet-derived growth factor receptor tyrosine kinase (PDGFR). The discovery of these drugs and many other kinase inhibitors in clinical development has been possible due in part to significant advances in the understanding of how they bind to their target kinases.

Structure-based drug design of protein kinase inhibitors

Structure-based drug design has played an important role in the rational design and development of small-molecule protein kinase inhibitor drugs. The catalytic or kinase domain of many protein kinases can be cloned and expressed in sufficient quantity and purity to allow for crystallization and elucidation of the three-dimensional structure. Since the publication of the first crystal structure of protein kinase A in 1991 [86, 87], a vast number of structures have been determined, covering most of the major families of protein kinases. A quick search for entries within the Research Collaboratory for Structural Bioinformatics Protein Data Bank (RCSB PDB) [88] reveals over 1000 protein kinase crystal structures. A high-resolution crystal structure, or especially a co-crystal structure with an inhibitor bound within the active site, is very useful for the rational design of subsequent chemical analogs of the inhibitor, to take advantage of potential binding site interactions. When used in an iterative approach, successive structural determinations and accompanying binding affinity data allow for a well defined structure-based drug design strategy for improving inhibitor potency. Selectivity can be improved within an inhibitor series by designing chemical analogs that favor binding interactions with the target protein kinase.

Another key consideration regarding protein kinase inhibitor design is the dynamic overall tertiary structure or conformational state of the protein. Protein kinases can adopt multiple conformational states, often dependent upon phosphorylation of specific residues. This was first demonstrated by crystallographic studies with the insulin receptor tyrosine kinase (INSR) as shown in Figure 4. The activation loop of the insulin-receptor kinase must be Tyr-phosphorylated in order for INSR to adopt an active conformation. In its nonactivated state, the activation loop (unphosphorylated) perturbs the binding of substrates, often referred to as the DFG-out conformation. Upon phosphorylation (either autophosphorylation or by another kinase), the activation loop moves into a productive conformation that allows ATP and protein or peptide substrates to bind [89, 90]. While not all protein kinases are regulated in the same phosphorylation-dependent DFG-in and DFG-out manner, the design of protein kinase inhibitors has often taken advantage of the conformational flexibility or plasticity to improve inhibitor potency and selectivity [91–93]. Some excellent reviews have explored the progress of structure-based drug design

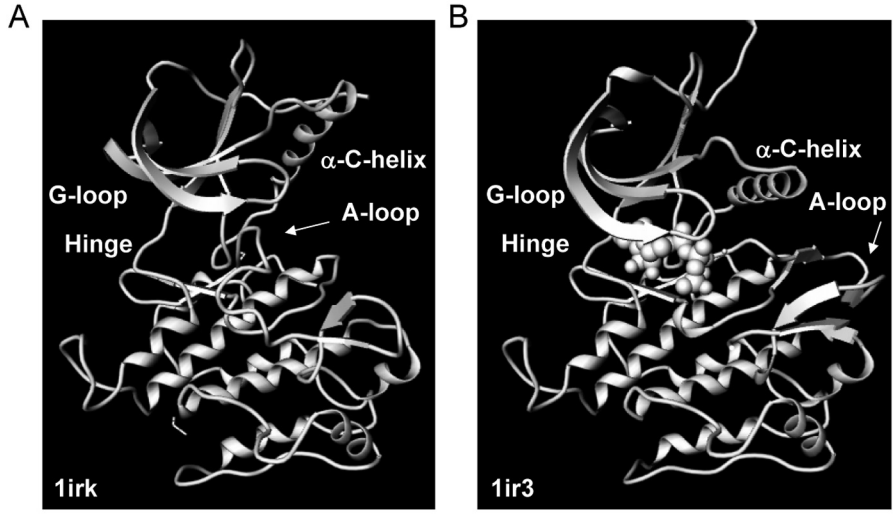

Figure 4. Crystal structure of the insulin receptor tyrosine kinase domain. Ribbon diagrams for *(A)* nonactivated (PDB code 1irk) and *(B)* activated (PDB code 1ir3) kinase. The activation loop (A-loop) is in the DFG-out conformation for the nonactivated form and in the open (DFG-in) conformation for the activated form. An ATP analog is shown (sphere representation) bound within the ATP site of the activated kinase.

for protein kinase inhibitors [94–97]. A few examples will be offered here to highlight the contributions of these approaches to the rational design and development of therapeutic protein kinase inhibitors.

Small-molecule inhibitors of protein kinases can be classified depending upon their mechanism of inhibition or mode of binding [97]. The classical protein kinase inhibitors bind within the ATP site in a competitive fashion, utilizing the purine nucleotide donor-acceptor binding motif to the protein hinge residues. Nonclassical inhibitors bind within the ATP site but also extend beyond the ATP pocket, making additional ligand interactions with the protein. Nonclassical inhibitors display a mixed competitive mechanism with respect to ATP. The classical and nonclassical inhibitor binding modes can be exemplified by the two therapeutic EGFR inhibitors, erlotinib (Tarceva) and lapatinib (Tykerb) (Figure 5). Erlotinib binds in a purely ATP-competitive fashion to EGFR and its co-crystal structure reveals that the compound overlays well with the binding of ATP (coordinated to hinge region and under the G-loop flap) [98]. In contrast, lapatinib binds within the ATP site and extends beyond to a "deep pocket" region that has opened by the movement of the alpha-C helix [99]. Some protein kinase inhibitors act by binding to and stabilizing the nonproductive forms of the kinase. For example, the therapeutic protein kinase inhibitors imatinib (Gleevec) and nilotinib (Tasigna) bind much more potently to the ABL tyrosine kinase in the nonactivated DFG-out conformation as illustrated in Figure 6 for the structure of imatinib bound to ABL [100].

All of the eight US FDA-approved, small-molecule inhibitors bind at or near the ATP pocket in either the classical or nonclassical manner. Yet, other protein kinase inhibitors can bind to other sites within the kinase domain or other regions of the protein (e.g., regulatory domains). The protein or peptide substrate binding site is another region of the kinase domain that can be utilized for structure-based drug design of inhibitors [101]. The clinical candidate KX01 (KX2–391, Kinex Pharma LLC) is an example of a nonpeptide inhibitor of c-Src tyrosine kinase (Src) that binds within the peptide substrate site and not the ATP site [102, 103]. An allosteric site has also been identified within the kinase domain of several protein kinases (e.g., ABL, p38 MAP kinase, MEK, and JNK) adjacent to the ATP site and amenable for small-molecule inhibitor design [93, 97]. Compounds that target this allosteric site are noncompetitive towards ATP and can form an inactive, ternary complex with the enzyme. The clinical candidates CI-1040 (PD184352, Pfizer) [104, 105], PD0325901 (Pfizer) [106], and ARRY-142886 (AZD6244, Array Biopharma/AstraZeneca) [107] represent allosteric inhibitors of MEK1, a Ser/Thr kinase within the RTK/RAF/MEK/ERK signaling pathway. Figure 7 shows the crystal structure of the ternary complex of MEK1 with ATP and PD-318088, a small-molecule inhibitor bound within the allosteric site [108]. The attractiveness of designing ATP-noncompetitive inhibitors targeted at this allosteric site is that they are expected to be independent of high cellular ATP concentration and potentially demonstrate greater selectivity towards inhibition of other protein kinases.

Protein kinase inhibitor selectivity profiling

Another advance has been the development of large protein kinase assay panels for the profiling of

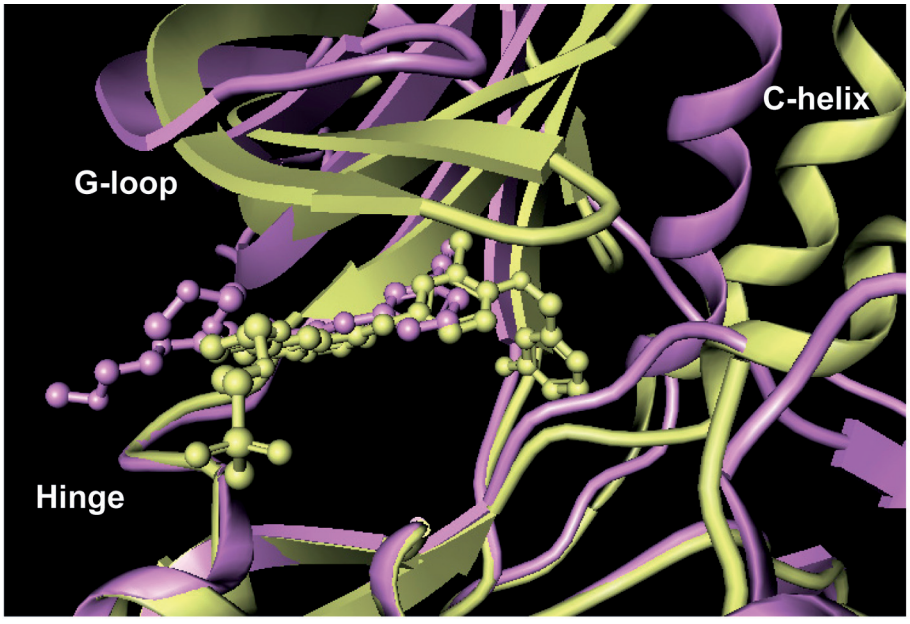

Figure 5. Crystal structures of EGRF kinase domain with Tarceva (eroltinib) and Tykerb (lapatinib). Overlay ribbon drawing of EGFR co-crystals with erlotinib (pink, PDB code 1 m17) and lapatinib (yellow, PDB code 1xkk) with inhibitors shown as ball-and-stick structures. Note that the fluoro-benzyloxy group of lapatinib binds deep within the ATP pocket formed by movement away from the alpha-C helix.

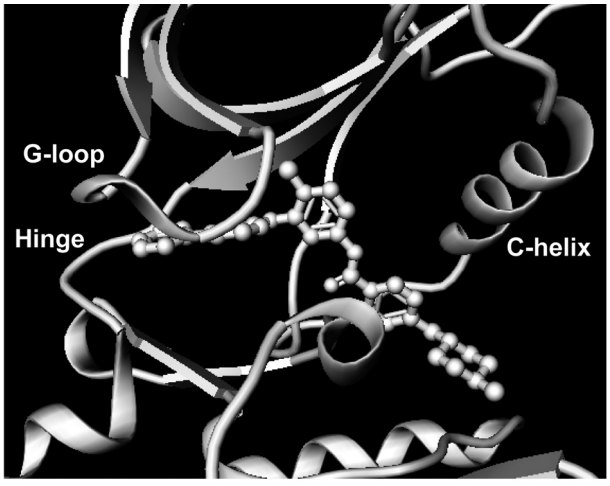

Figure 6. Crystal structure of c-ABL with Gleevec (imatinib). Ribbon representation of c-ABL protein co-crystallized with imatinib (PDB code 1iep). Imatinib (ball-and-stick) binds in an extended conformation spanning the ATP site (to hinge region under G-loop) and into the DFG-out binding pocket where the methyl-piperizine portion of the inhibitor resides.

inhibitors in terms of their selectivity. Kinase selectivity screening of inhibitors provides information on the potential inhibitory activity of a compound across a broad range of protein kinases and their respective signaling pathways. Scientists from the Cohen labs at the University of Dundee [109, 110] and elsewhere [111–113] have tested numerous kinase inhibitors against large kinase activity panels. In some instances, inhibitors were found to be considerably less selective than originally reported.

A number of life science companies and research labs offer the profiling of compounds against large kinase screening panels. They are often able to test compounds against hundreds of different protein kinases [114, 115]. These large protein kinase panels typically utilize recombinant enzymes and perform biochemical activity assays with either protein or peptide substrates. Life science vendors that offer this activity-based type of kinase inhibitor selectivity screening service include: Caliper LifeSciences (Rapid KinaseAdvisor™), Carna Biosciences (BioFocus®), Invitrogen (SelectScreen™), MDS Pharma Services (FastKinase™), Millipore (KinaseProfiler™), ProQinase GmbH (iProKiTe®), Reaction Biology Corp. (Kinase HotSpot^{SM}), Shanghai ChemPartner, and SignalChem. In addition, several novel screening technologies have been developed that measure the binding of inhibitors to both the activated and nonactivated forms of protein kinases. Through the use of kinase-directed affinity ligands, Ambit Biosciences and ActivX Biosciences are life science laboratories that profile the binding affinity of compounds. The Ambit KINOMEscan™ profiling technology utilizes recombinant wild-type and mutant kinases from phage display to evaluate the ability of a drug to competitively displace kinases from immobilized affinity ligand probes. Only those kinases that bind the drug will be displaced and these displaced kinases represent a unique selectivity profile for that compound [111, 113]. The ActivX KiNativ™ platform uses irreversible affinity probes such as biotinylated acyl phosphate ATP to capture protein kinases from biological samples [116]. This innovative technology can evaluate an ATP-competitive compound against active and nonactivated kinases, pseudokinases [1], and other ATP-binding proteins. It should be noted that

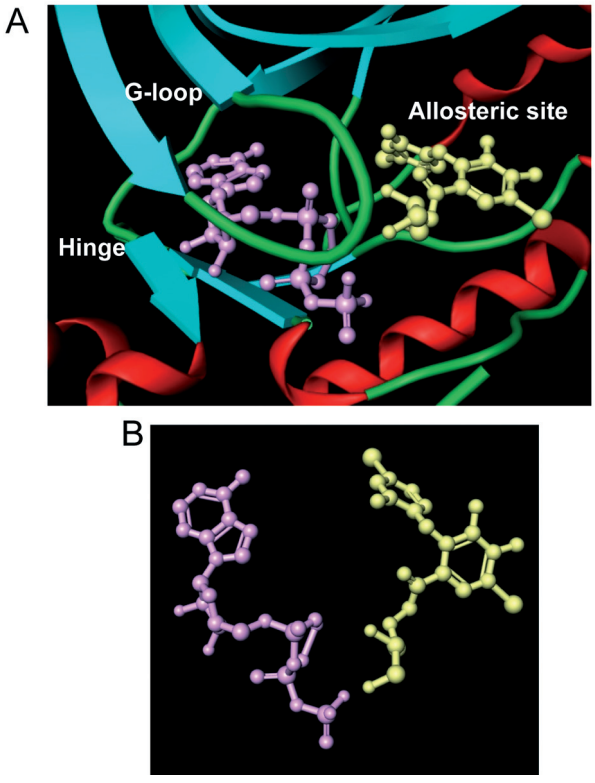

Figure 7. Crystal structure of MEK1 with allosteric inhibitor. MEK1 ternary complex structure. *(A)* Ribbon representation of MEK1 co-crystal structure (PDB code 1 s9j) with ball-and-stick ligands: Mg-ATP (pink) under G-loop and inhibitor PD0318088 (yellow) in allosteric site. *(B)* Same ternary structure slightly rotated, but the protein is hidden to show non-overlapping binding of ATP (pink) and PD0318088 (yellow).

further follow-up in cellular or animal models is required to better understand the physiological relevance of the results from these biochemical kinase selectivity screens.

Since specific oncogenic kinases may drive the proliferation of tumors, a concept often referred to as oncogene addiction [117, 118], some kinase inhibitors may only exhibit tumor growth inhibition against cancers that express certain oncogenic or mutant kinases (e.g., EGFR). The inhibitory activity of some therapeutic RTK inhibitors against multiple protein kinases may be important for their therapeutic efficacy, such as the inhibition of KIT by imatinib [73], dasatinib [75], and sunitinib [82, 83]. Understanding which specific kinase or group of kinases is inhibited can help identify which cancers will be best treated by that drug. Accordingly, kinase selectivity screening has become increasingly important in the development of therapeutic protein kinase inhibitors.

Protein kinase inhibitor safety considerations

While advances in protein kinase drug discovery continue and many protein kinase inhibitors (both biologic and small-molecule) remain in clinical trials, so far the overall medical promise of this class of drugs has been limited to only a small number of approved drugs. This may be attributable in part to the nature of the therapeutic disease areas that are targeted by kinase inhibitors. For instance, cancer is an intrinsically mutagenic disease with a high rate of tumor resistance to single drug therapy. While oncogenic kinases derived from somatic mutation or chromosomal alteration may drive the growth of some tumors [119], specific activating mutations may only reside in tumors from small subgroups of patients. This accounts for the low response rates observed in early clinical studies for the EGFR inhibitor gefitinib with unselected non-small cell lung cancer patients, where the clinical benefit correlated with specific EGFR mutations [120, 121]. Likewise, tumor multi-drug resistance mechanisms and acquired resistance to kinase inhibitors, such as EGFR inhibitors, can limit initial drug response by development of secondary EGFR mutations or amplification of additional RTKs [117, 118, 122].

As a class of molecularly targeted therapies for the treatment of cancer, protein kinase inhibitors have made a substantial beneficial impact on the therapeutic care of cancer patients. They have provided a new treatment paradigm and greatly improved the quality of life for patients with advanced cancer and poor prognosis. Protein kinase inhibitors are usually well tolerated and have shown an overall better safety profile than cytotoxic chemotherapies, with toxicities and side effects that are generally more manageable and reversible [123, 124]. Some of the safety issues associated with therapeutic biologic and small-molecule protein kinase inhibitors, particularly potential cardiovascular and dermatological toxicities, will be reviewed here.

The first therapeutic protein kinase inhibitor to be launched was Herceptin (trastuzumab) in 1998 by Genentech. Trastuzumab is a monoclonal antibody that targets ErbB-2 (HER2), an RTK that is overexpressed in a significant number of breast cancers [19, 20]. Patients treated with trastuzumab have a small but increased risk of cardiac dysfunction [18, 20]. Further research into potential mechanisms for the cardiotoxicity of ErbB-2 (HER2) inhibition found that conditional mutation of ErbB-2 in transgenic mice caused progressive heart malfunctions [125]. In addition, treatment of rat cardiomyoctes with anti-ErbB2 antibodies caused increased mitochondrial dysfunction and cellular apoptosis [126]. Although

the relevance of these *in vitro* studies to the clinical observations is unclear, these results suggest that the cardiac toxicity associated with trastuzumab might be mechanism-based and directly attributed to ErbB-2 inhibition. In contrast to the cardiomyopathy associated with trastuzumab treatment, cardiotoxicity has not nearly been as prevalent with lapatinib (Tykerb), a small-molecule inhibitor that also targets ErbB-2 [127]. Lapatinib is a dual specificity inhibitor that is potent against both ErbB-2 and EGFR. Indeed, the most common side effects reported for lapatinib treatment are more closely related to those observed for other EGFR inhibitors [127].

Several recent reports have also indicated cardiotoxicity as a risk factor for other therapeutic kinase inhibitors, including imatinib [128], sunitinib [129, 130], and sorafenib [131], although these side effects have been effectively managed for most patients [132]. For imatinib treatment, left ventricular dysfunction and congestive heart failure were observed in some patients under treatment [128]. Yet, in a later retrospective analysis of imatinib-treated patients, the incidence of congestive heart failure was deemed to be rare without pre-existing cardiac conditions [133]. A report concerning the effect of imatinib on mouse cardiomyocytes in culture proposed that the inhibition of ABL and activation of the endoplasmic reticulum stress response were potential mechanisms for the observed cardiomyocyte toxicity [128]. Congestive heart failure has not been cited as a common adverse event for the ABL inhibitors, dasatinib [134] or nilotinib [135], although other cardiac toxicities such as QT interval prolongation have been reported [132, 136]. The most common adverse events reported for these therapeutic BRC-ABL inhibitors were myelosuppression and neutropenia [132, 134, 136].

Several recent studies have underscored the need to monitor cardiac function in patients treated with sunitinib (multi-RTK inhibitor) due to an unanticipated risk for congestive heart failure [129, 130]. Some cardiovascular effects have been previously observed in sunitinib trials [136] and are included in the approved product labeling along with recommendations for monitoring. Force et al. [136] proposed that cardiomyocytes may be more sensitive to RTK inhibitors since these cells have a high demand for ATP and may be more susceptible to mitochondrial effects from targeted kinase inhibitors. In addition, hypertension has been recognized as a potential side effect of VEGFR inhibitors like sunitinib and sorafenib and might therefore contribute to the observed cardiovascular abnormalities [137]. Hypertension has also been observed with other anti-VEGF therapies, including the therapeutic monoclonal antibody bevacizumab (Avastin) [138].

For the therapeutic EGFR-targeted inhibitors gefitinib, erlotinib, lapatinib, cetuximab (Erbitux), and panitumumab (Vectibix), the most frequently associated adverse events are fatigue, diarrhea, and the development of dermatological toxicities, acneiform-like rash and hand-foot syndrome [139, 140]. As yet, the pathogenesis of the skin rash is unclear, but it appears that it is a mechanism-based toxicity and may result from inhibition of EGFR within the keratinocytes, possibly causing a subsequent inflammatory response [139, 140]. While treatments are being pursued to minimize the deleterious effects of the skin rash and to improve patient compliance, the development of the rash itself is a potential biomarker for EGFR inhibition. In fact, studies have shown a good correlation between the occurrence of the rash during erlotinib or cetuximab treatment and their clinical benefit [141, 142]. Other RTK inhibitors such as sunitinib and sorafenib also have the potential of cutaneous toxic side effects of dry skin and rash [143]. Although skin toxicity is not a very common side effect for anti-VEGF therapy, recently a correlation was also made between the development of rash during bevacizumab treatment and a positive response in patients with metastatic colorectal cancer [144, 145].

The clinical development of therapeutic protein kinase inhibitors for diseases other than cancer has also been advancing. The development of inhibitors of p38 mitogen-activated protein kinase (MAPK), a Ser/Thr kinase, exemplifies an area of intense activity by pharmaceutical companies, yet without any approved agents to date [146]. The stress-activated p38 MAPK signaling pathway has been well validated as an important target for the discovery of anti-inflammatory agents, mostly through down-regulation of the tumor necrosis factor α (TNF-α) (see Figure 1). A number of clinical p38 MAPK inhibitors have emerged for inflammatory disease indications such as rheumatoid arthritis, but most have failed due to lack of target modulation, adverse events and toxicities, or poor pharmacokinetics [147, 148]. Examples of p38 MAPK inhibitors tested in clinical studies include BIRB-796 (doramapimod, Boehringer Ingelheim), VX-745 (Vertex), and SCIO-469 (talmapimod, Scios/J&J) (Figure 8). Both BIRB-796 and VX-745 were discontinued due to hepatotoxic elevation of liver transaminases, skin rash, and other adverse events [149, 150]. Second generation p38 MAPK inhibitors such as VX-702, have been developed to address some of these toxicity and pharmacokinetic issues. Recently, mild-to-moderate adverse events including skin rash and dose-dependent QT interval prolongation were observed in a phase II study of VX-702 as a monotherapy or in combination with metho-

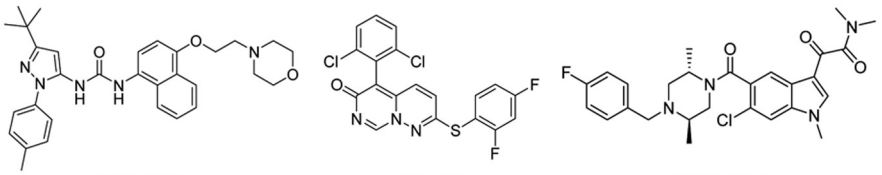

Figure 8. Structures of some p38 MAPK inhibitors in clinical trials.

trexate for rheumatoid arthritis [151]. Some innovative strategies have also been employed to address safety and pharmacokinetic issues for p38 MAPK inhibitors. Tissue-specific drug delivery and formulation technologies have been investigated, such as lysosome-conjugation of a p38 MAPK inhibitor to render it more renal specific [152]. Promising results have been reported for substrate selective inhibitors of p38 MAPK that prevent phosphorylation and activation of the downstream substrate MK2 but not the activating transcription factor 2 (ATF2) pathway [153]. This type of selective blocking of the p38 MAPK signaling pathway might achieve the desired pharmacological effect but avoid modulating the MAPK-dependent signaling associated with the skin rash side effect.

Overall, therapeutic protein kinase inhibitors for cancer treatment have shown better tolerability than earlier cytotoxic drugs. Yet, the commonality of observed cardiac and skin-related toxicities for these targeted therapeutics, even if infrequent and manageable, suggests a possible class-specific response. Further investigations are therefore warranted to determine whether or not these toxicities are class-specific for Tyr-kinase inhibitors. It is yet to be clearly determined if similar adverse side effects will be observed with other kinase inhibitors, such as Ser/Thr kinase inhibitors, which are still progressing through clinical trials.

Closing comments

It has been nearly half a century since the discovery by Fischer and Krebs of reversible protein phosphorylation and the initial recognition of its regulatory function in cellular pathways. Since then, researchers have gained a greater understanding of the diverse cellular processes regulated by protein kinases and their pathogenic role in various diseases. This is highlighted by some major research advances such as the discovery that oncogenic kinases have a role in many cancers, the complete mapping of the human kinome, and the development of the first therapeutic protein kinase inhibitors, Herceptin and Gleevec. Kinase drug discovery research continues to advance through the application of innovative design strategies and novel technologies such as those highlighted in this review. These advances have allowed kinase drug researchers to overcome much of the early skepticism over achievable kinase inhibitor potency and selectivity. And more importantly, these advances have resulted in the generation of a new class of targeted anti-cancer drugs that have benefited patients and provided an improvement in tolerability over earlier cytotoxic chemotherapies. Yet, as the next generation of protein kinase inhibitors progresses through clinical studies, researchers and clinicians must continue to properly balance the value and therapeutic benefit of these drugs and their potential safety risks.

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