MINIREVIEW

25 Years of Small Molecular Weight Kinase Inhibitors: Potentials and Limitations

Doriano Fabbro
PIQUR Therapeutics AG, Basel, Switzerland

ABSTRACT

Deregulation of protein and lipid kinase activities leads to a variety of pathologies, ranging from cancer inflammatory diseases, diabetes, infectious diseases, and cardiovascular disorders. Protein kinases and lipid kinases represent, therefore, an important target for the pharmaceutical industry. In fact, approximately one-third of all protein targets under investigation in the pharmaceutical industry are protein or lipid kinases. To date, 30 kinase inhibitors have been approved, which, with few exceptions, are mainly for oncological indications and directed against only a handful of protein and lipid kinases, leaving 70% of the kinase untapped. Despite these successes in kinase drug discovery, the development of kinase inhibitors with outstanding selectivity, identification and validation of driver kinase(s) in diseases, and the emerging problem of resistance to the inhibition of key target kinases remain major challenges. This minireview provides an insight into protein and lipid kinase drug discovery with respect to achievements, binding modes of inhibitors, and novel avenues for the generation of second-generation kinase inhibitors to treat cancers.

Introduction

Protein and lipid kinases represent, after GTP protein-coupled receptors and proteases, one of the most important target classes for treating human disorders. A set of divergent kinases play essential roles in eukaryotic signaling. These include various protein kinases like BRAF, ABL, ALK, as well as kinases that are able to phosphorylate phosphatidyl-inositol (PI) like PI3Ks and PI4Ks (Engelman, 2009; Courtney et al., 2010). Many human malignancies are associated with deregulated activation of protein or lipid kinases due to mutations, chromosomal rearrangements, and/or gene amplification (Hanahan and Weinberg, 2000; Hunter, 2000; Blume-Jensen and Hunter, 2001; Cohen, 2002; Hahn and Weinberg, 2002; Weinstein, 2002; Bardelli et al., 2003; Levitzki, 2003; Vieth et al., 2004, 2005; Luo et al., 2009; Fedorov et al., 2010; Fabbro et al., 2011).

The human kinome contains typical, atypical protein kinases, as well as pseudo-kinases (Manning et al., 2002; http://kinase.com/kinbase). The latter makes up 10% of human protein kinases and are either inactive or only weakly active due to the lack of at least one of three motifs in the catalytic domain that are essential for catalysis (Boudeau et al., 2006; Kannan and Taylor, 2008). Despite lacking the ability to phosphorylate substrates, pseudo-kinases are still very important in regulating diverse cellular processes, as indicated by the regulation of LKB1 by STRAD and the activation of JAK2 by V617F on its JH2 pseudo-kinase domain (Boudeau et al., 2006; Kannan and Taylor, 2008).

Protein kinases have a bilobal architecture, with one N-terminal lobe with mainly β-sheets and one C-terminal domain with α helices. The two lobes are connected by the hinge region, which lines the ATP-binding site, which is targeted by the majority of small molecular weight kinase inhibitors (Cohen, 2002; Levitzki, 2003; Vieth et al., 2004, 2005; Cowan-Jacob, 2006; Fabbro et al., 2011). In past decades, 29 small-molecule kinase inhibitors have been approved for clinical use, mainly for oncological indications. Despite these successes, three major challenges in kinase drug discovery remain:

1. Kinase inhibitors with outstanding selectivity are likely to become important not only for minimizing side effects and allowing chronic treatment of non-life-threatening diseases, but also to better understand the on- and off-target pharmacology of kinase inhibitors (Noble et al., 2004; Fabbro et al., 2011; Cowan-Jacob et al., 2014).

2. Identification and validation of the driver kinase(s) in human malignancies and disorders (Weinstein, 2002; Luo et al., 2009) by genome-wide screening for kinase amplifications/translocations/mutations in conjunction

ABBREVIATIONS: CML, chronic myeloid leukemia; EGFR, epidermal growth factor receptor; FGFR, fibroblast growth factor receptor; myr, myristate; PI, phosphatidyl-inositol; PX-866, 1E,4S,4aR,5R,6aS,9aR)-5-(acetyloxy)-1-[(di-2-propen-1-ylamino)methylene]-4,4a,5,6,6a,8,9,9a-octahydro-11-hydroxy-4-(methoxymethyl)-4a,6a-dimethyl-cyclopenta[5,6]naphtho[1,2-c]pyran-2,7,10(1H)-trione; SSR128129E, benzoic acid, 2-amino-5-[1-methoxy-2-methyl-3-indoliziny]carbonyl]-, sodium salt.

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with proteomic technologies. These studies have revealed how the mutational status of kinases may be associated with various cancer conditions (Hunter, 2000; Blume-Jensen and Hunter, 2001; Cohen, 2002; Bardelli et al., 2003; Sawyers, 2004; Vieth et al., 2004; Takano et al., 2005; Ventura and Nebreda, 2006; Wolf-Yadlin et al., 2006; Ali and Ali, 2007; Engelman et al., 2007; Greenman et al., 2007; Thomas et al., 2007).

3. Emerging resistance to the inhibition of key target kinases. Multiple mechanisms of resistance have been and are being elucidated to improve the efficacy of these types of targeted therapies.

In this review, we will update the current status of kinase drug discovery and discuss strategies of how to override these various types of resistances, including compounds capable of circumventing the target related drug resistance (Lombardo et al., 2004; Weisberg et al., 2005; Adrian et al., 2006; Quintas-Cardama et al., 2007; Engelman et al., 2008b; Zhang et al., 2010).

**Current Status of Protein and Lipid Kinase Drug Discovery**

**Protein Kinase Domain.** Thanks to the many available structures of kinase domains, we have a reasonable understanding of the various structural elements that are required for the phosphorylation reaction (Cowan-Jacob et al., 2014). Protein kinase domains consist of a small, mostly β-structured N-lobe connected by a short hinge fragment to a larger α-helical C-lobe. ATP binds in the cleft between the N- and C-terminal lobes of the kinase domain where the adenine group of ATP is sandwiched between hydrophobic residues and makes contact via hydrogen bonds to the hinge (Nolen et al., 2004; Taylor and Kornev, 2011; Fabbro et al., 2011). Just N-terminal of the hinge, deep in the ATP pocket, is an important residue called the “gatekeeper”, which is often mutated in kinase alleles resistant to inhibitors. The gatekeeper controls the access to the hydrophobic “back pocket” of the kinase (Nolen et al., 2004; Kornev et al., 2006; Cowan-Jacob et al., 2009; Taylor and Kornev, 2011; Moebitz and Fabbro, 2012). An outstanding structural element of the ATP-binding sites is the activation loop (also called the activation segment or A-loop), with its N-terminal Asp-Phe-Gly (DFG) motif, whose orientation is pivotal for the access of the protein substrate sites (Nolen et al., 2004; Cowan-Jacob, 2006). Other elements required for catalysis are the C-helix, which contains the Glu that forms a salt bridge with the active site Lys (the Ala-X-Lys motif in the β3-strand), thereby anchoring and orienting the ATP, P-loop (a Gly-rich loop also known as the G-loop), which contributes to coordination of the phosphates of ATP, catalytic loop (the YHRD motif in the β6/β7-strand), where Asp functions as a base acceptor for the proton transfer, and finally, the abovementioned DFG motif, where Asp binds the Mg2+ ions that coordinate the β-and γ-phosphates of ATP in the ATP-binding cleft (Hanks and Hunter, 1995; Nolen et al., 2004; Cowan-Jacob, 2006; Taylor and Kornev, 2011).

**Approved Kinase Inhibitors.** Since the approval of fasudil in 1995, sirolimus in 1999, and imatinib in 2001, the number of approved kinase inhibitors has increased to 30, with many others still in the preclinical state (Table 1). More than 130 kinase inhibitors are reported to be in phase 1–3 clinical trials (http://www.clinicaltrials.gov/) (Vieth et al., 2005). It is beyond the scope of this review to discuss all of the protein kinase inhibitors that are in preclinical or clinical development. Although there are many more kinase inhibitors in development, it should be emphasized that all of the mentioned approved and advanced kinase inhibitors do not cover more than 10–15% of the whole kinome (Fedorov et al., 2010).

With a few exceptions, like the rapalogs (everolimus and temsirolimus) and tramefinib, all of the small molecular weight kinase inhibitors are directed toward the ATP-binding site. The conservation of ATP binding in the human kinome causes these ATP mimics to often crossreact with many other different kinases, resulting in compounds with promiscuous profiles like, for example, dasatinib (Lombardo et al., 2004) or sunitinib (Motzer et al., 2006; Faiivre et al., 2007). The relative restrictions imposed by the ATP pharmacophore make it increasingly difficult to operate in a free intellectual property space and has increased the interest in identifying inhibitors that do not compete with ATP (Fabbro et al., 2011; Moebitz and Fabbro, 2012; Cowan-Jacob et al., 2014).

Most of the approved kinase drugs are active against more than one type of cancer. Only a few of them have been used for the treatment of nononcological indications, namely, tofacitinib for rheumatoid arthritis, nitedanib for idiopathic pulmonary fibrosis, everolimus for organ rejection of the heart and kidney, and fasudil for cerebral vasospasm (Table 1).

Also, most of the approved kinase drugs are active against more than one type of cancer. On the other hand, there are multiple kinase drugs for a single indication. For example imatinib, nilotinib, dasatinib, bosutinib, and ponatinib have all been approved for chronic myeloid leukemia (CML), while sorafenib, sunitinib, everolimus, temsirolimus, axitinib, or pazopanib are indicated for various stages of renal cell cancer. Ceritinib and crizotinib are indicated for non–small-cell lung cancer with ALK translocations, while gefitinib, erlotinib, and afatinib are indicated for non–small-cell lung cancer with an activated epidermal growth factor receptor (EGFR). Vandetanib and cabozantinib are used for the treatment of medullary thyroid carcinoma, while imatinib, sunitinib, and regorafenib are indicated for gastrointestinal tumors. Finally, vemurafenib alone or the combination of dabrafenib with trametinib is indicated for metastatic melanoma with BRAFV600 mutations (Table 1).

The actual landscape of kinase inhibitor drugs developed over the last two decades shows that only a handful of protein kinases (about 10% of the kinome) have been successfully targeted with inhibitors, which mainly target oncological indications. It should be emphasized that selective protein kinase inhibitors will not only be important for the treatment of diseases but also as useful reagents to better understand the on- and off-target kinase biology (Robert et al., 2005; Force et al., 2007; Knapp et al., 2013).

**Binding Modes of Kinase Inhibitors**

Kinase inhibitors show different modes of binding to kinases. In principle, one can divide kinase inhibitors into those that bind covalently or reversibly to the kinase (Zhang et al., 2009; Cowan-Jacob et al., 2014).

**Covalent Inhibitors**

Inhibitors that bind covalently may have reversible or irreversible binding modes, depending on the reactivity of the
war-head (Cowan-Jacob, 2006; Zhang et al., 2009; Liu et al., 2013). Many inhibitors bind covalently to the ATP site to form a bond to a Cys residue in or around the active site, preventing the binding of ATP to the protein kinase (Zhang et al., 2009; Liu et al., 2013). The most prominent example is the recently approved BTK inhibitor ibrutinib, which targets the Cys in the bottom part of the ATP-binding site [receptor protein tyrosine kinase (RTK)] (Zhou et al., 2009, 2011; Wiestner, 2013; Akinleye et al., 2014).

More covalent inhibitors targeting these cysteines are being developed to target the most resistant form of the EGFR RTK that emerges upon treatment with noncovalent EGFR inhibitors (Zhang et al., 2009; Zhou et al., 2011; Liu et al., 2013). An early discovered type of covalent binder is exemplified by Wortmannin (or its derivative PX-866 [1E,4S,4aR,5R,6aS, 9aR]-5-(acetyloxy)-1-[(di-2-propen-1-ylamino)methylene]-4,4,5,6,6-

### TABLE 1
Approved kinase inhibitors as of October 2014

<table>
<thead>
<tr>
<th>Generic Name (Compound Code, Trade Names)</th>
<th>Kinase Target</th>
<th>Disease</th>
<th>Company (Year, Type)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tasocitinib (INC424, Jakafi)</td>
<td>JAK2</td>
<td>IMF with JAK2V617F mutations</td>
<td>Novartis, Incyte (2011, type 1)</td>
</tr>
<tr>
<td>Axitinib (AG013736, Inlyta)</td>
<td>VEGFR, KIT, PDGFR, RET, CSFIR, FLT3</td>
<td>RCC</td>
<td>Pfizer (2012, type 1)</td>
</tr>
<tr>
<td>Regorafenib (BAY 73-4506, Stivarga)</td>
<td>VEGFR2, Tie2</td>
<td>CRC, GIST</td>
<td>Bayer (2012, type 2)</td>
</tr>
<tr>
<td>Pazopanib (GW-760304, Votrient)</td>
<td>VEGFR, PDGFR, KIT, JAK3</td>
<td>RCC</td>
<td>GSK (2012, type 1)</td>
</tr>
<tr>
<td>Tofacitinib (CP-690550, Xeljanz, Tasocitinib)</td>
<td>VEGFR2, PDGFR, KIT, FLT3</td>
<td>MTC</td>
<td>Pfizer (2012, type 1)</td>
</tr>
<tr>
<td>Ponatinib (AP24534, Iclusig)</td>
<td>ABL</td>
<td>Imatinib-resistant CML with T315I mutations</td>
<td>Novartis, Incyte (2011, type 1)</td>
</tr>
<tr>
<td>Bosutinib (SKI-606, Bosulif)</td>
<td>ABL</td>
<td>Metastatic melanoma with BRAFV600E mutations</td>
<td>Pfizer (2012, type 1)</td>
</tr>
<tr>
<td>Dasatinib (AMN107, Tasigna)</td>
<td>ABL, PDGFR, KIT, SRC</td>
<td>RCC</td>
<td>Pfizer (2012, type 1)</td>
</tr>
<tr>
<td>Everolimus (RAD001, Afinitor, Votrient)</td>
<td>mTOR</td>
<td>RCC</td>
<td>Pfizer (2009, type 3)</td>
</tr>
<tr>
<td>Temsirolimus (CCI-779, Torisel)</td>
<td>MET and ALK</td>
<td>NSCLC with ALK translocations</td>
<td>Pfizer (2011, type 1)</td>
</tr>
<tr>
<td>Vemurafenib (PLX4032, RG7204, Zelboraf)</td>
<td>BRAF</td>
<td>Metastatic melanoma with BRAFV600E mutations</td>
<td>Novartis, Incyte (2011, type 1)</td>
</tr>
<tr>
<td>Cabozantinib (XL184, BMS90751, Cometriq)</td>
<td>VEGFR, PDGFR, KIT, FLT3</td>
<td>MTC</td>
<td>Pfizer (2012, type 1)</td>
</tr>
<tr>
<td>Nintedanib (BIBF 1120, Vargatef, intedanib)</td>
<td>VEGFR, PDGFR, FGFR</td>
<td>Idiopathic pulmonary fibrosis</td>
<td>Pfizer (2012, type 1)</td>
</tr>
<tr>
<td>Sorafenib (BAY 43-9006, Nexavar)</td>
<td>VEGFR2, PDGFR, KIT, FLT3, BRAF</td>
<td>RCC, HCC</td>
<td>Roche, OSI (2004, type 1)</td>
</tr>
<tr>
<td>Gefitinib (ZD1839, Iressa)</td>
<td>EGFR</td>
<td>NSclc, pancreatic cancer</td>
<td>Onyx (2005, type 2)</td>
</tr>
<tr>
<td>Erlotinib (OSI-774, Tarceva)</td>
<td>EGFR</td>
<td>NSCLC, pancreatic cancer</td>
<td>Pfizer (2005, type 1)</td>
</tr>
<tr>
<td>Imatinib (STI571, Gleevec, Gleevec)</td>
<td>ABL, PDGFR, KIT, SRC</td>
<td>CML</td>
<td>Pfizer (2005, type 1)</td>
</tr>
<tr>
<td>Laptatinib (GW2016, Tykerb)</td>
<td>EGFR, ERBB2</td>
<td>BC</td>
<td>Pfizer (2009, type 3)</td>
</tr>
<tr>
<td>Lapatinib (GW-54485, Spryce)</td>
<td>ABL, PDGFR, KIT, SRC</td>
<td>CML</td>
<td>Pfizer (2009, type 3)</td>
</tr>
<tr>
<td>Gefitinib (ZD1839, Iressa)</td>
<td>EGFR</td>
<td>NSCLC, pancreatic cancer</td>
<td>Pfizer (2005, type 1)</td>
</tr>
<tr>
<td>Fasludil (HA-1077)</td>
<td>ROCK</td>
<td>Cerebral vasospasm, PAH</td>
<td>Pfizer (2005, type 1)</td>
</tr>
<tr>
<td>Sirolimus (Rapamune)</td>
<td>mTOR</td>
<td>Kidney transplants</td>
<td>Pfizer, Wyeth (1999, type 3)</td>
</tr>
</tbody>
</table>

**AZ, Astra-Zeneca; BC, breast cancer; BI, Boehringer-Ingelheim; BMS, Bristol-Myers Squibb; CLL, chronic lymphocytic leukemia; CMML, chronic mononuclear leukemia; CRC, colorectal cancer; FL, follicular lymphoma; HCC, hepatocellular carcinoma; HES, hyper-eosinophilic syndrome; IMF, interstitial myelofibrosis; GIST, gastrointestinal stromal tumor; GSK, Glaxo-Wellcome; MCL, mantle cell lymphoma; MTC, medullary thyroid cancer; PAH, Pulmonary Arterial Hypertension; Ph+, B-ALL, Philadelphia chromosome positive B-cell acute lymphoblastic leukemia; RA, rheumatoid arthritis; RCC, renal clear cell carcinoma; SEGA, subependymal giant cell astrocytoma; SLL, small lymphocytic leukemia.**
which binds to the active site Lys in the ATP-binding site of the PI3K (Wymann et al., 1996; Ihle et al., 2004). Various covalent inhibitors targeting different cysteines in various kinases have been reported (Liu et al., 2013; Kwiatkowski et al., 2014).

Reversible (Noncovalent) Inhibitors

Noncovalent kinase inhibitors can be further classified into those which either bind or do not bind to the hinge region of the kinase, leading to the classification of type 1–3 reversible kinase inhibitors (Liu and Gray, 2006; Zhang et al., 2009). These have been briefly summarized below.

Type 1 and 1.5 Inhibitors. These inhibitors fulfill all of the criteria of ATP mimetics by binding to the hinge and targeting an active conformation of the kinase (often referred to as DFG-in). In the active kinase, the A-loop adopts an open conformation typical for the ATP-bound state of the kinase where the Asp in the DFG motif coordinates the phosphates of ATP, while Phe stabilizes helix C and A-loop for catalysis (Nolen et al., 2004; Cowan-Jacob, 2006; Liu and Gray, 2006; Zhang et al., 2009; Cowan-Jacob et al., 2014). Many of the type 1 inhibitors use variation in the size, shape, and polarity of the gatekeeper residue to gain selectivity and knowledge about the various inactive conformations of different kinases, which allows one to combine these features (Liu and Gray, 2006; Cowan-Jacob et al., 2014). The bias toward kinase inhibitors that target the active conformation of the kinase stems from the early days of kinase drug discovery, where an ATP mimetic design, together with the use of enzymatic assays displaying the highest level of activity, was used. Classic examples for this type of approved kinase inhibitor class are gefitinib, erlotinib, dasatinib, and sunitinib (Table 1).

The type 1.5 inhibitor is a subtype of the type 1 inhibitor that binds to an inactive kinase conformation (Nolen et al., 2004; Cowan-Jacob, 2006; Liu and Gray, 2006; Zhang et al., 2009; Cowan-Jacob et al., 2014). Lapatinib in the EGFR-RTK adopts a DFG-in conformation, which is typical of an active kinase, but helix C is pushed out by lapatinib, effectively disrupting the ion pairing between the active site Lys and the Glu from helix C (Wood et al., 2004). This type of binding to the DFG-in inactive conformation, which is referred to as type 1.5 inhibition, has also been observed in other kinases (Cowan-Jacob et al., 2014).

Although the structures of protein kinases in the active ATP-bound state are very similar, specificity can be gained through particular features in the ATP-binding sites of kinases. More selective type 1 inhibitors are those that, like the type 1.5 inhibitors, use additional sites close to the ATP binding, like the adjacent hydrophobic pockets whose entry is regulated by the gatekeeper (Zuccotto et al., 2010). Other type 1 inhibitors interact with two different sites on the kinase, such as the ATP site and the peptide-binding site like the bivalent/bitopic inhibitors (Hill et al., 2012), macrocycles (Tao et al., 2007), or some of the covalent inhibitors (Liu et al., 2013).

Type 2 Inhibitors. Type 2 kinase inhibitors bind to the inactive, so-called “DFG-out conformation”, maintaining contacts to the hinge region and usually displaying an ATP-competitive behavior similar to type 1 inhibitors (Nolen et al., 2004; Cowan-Jacob, 2006; Liu and Gray, 2006; Zhang et al., 2009). The transition of the active DFG-in to the inactive DFG-out conformation exposes an additional hydrophobic pocket adjacent to the ATP site, which is used by type 2 inhibitors, locking the kinase in the inactive conformation (Nolen et al., 2004; Cowan-Jacob, 2006; Liu and Gray, 2006; Zhang et al., 2009; Cowan-Jacob et al., 2014). Approved kinase inhibitors binding to or stabilizing the DFG-out conformations are, for example, imatinib, nilotinib, or sorafenib (Table 1).

In addition to DFG-out, combinations of different conformational states of helix C, the A-loop, and/or P-loop can generate various inactive conformations of the kinase domain (Cowan-Jacob, 2006; Cowan-Jacob et al., 2014). Each individual kinase has a preferred inactive conformation, depending on its phosphorylation state and regulatory mechanisms involving structures outside the kinase domain (Cowan-Jacob et al., 2014).

Type 3 Inhibitors. Type 3 kinase inhibitors are non-ATP competitive (allosteric) kinase inhibitors that have no physical contact with the hinge. As they exploit binding sites and regulatory mechanisms that are unique to a particular kinase, they therefore show the highest degree of selectivity (Nolen et al., 2004; Cowan-Jacob, 2006; Liu and Gray, 2006; Zhang et al., 2009; Cowan-Jacob et al., 2014). The noncatalytic role of kinases involving unique nonconserved interactions and which increase the target space on the kinase have only recently been appreciated (Rauch et al., 2011; Cowan-Jacob et al., 2014).

Type 3 inhibitors can either bind to the kinase domain (close to or removed from the ATP site) or to sites that are located outside of the kinase domain. These type 3 inhibitors include very diverse compounds, ranging from the MEK inhibitors to rapamycin derivatives.

The allosteric inhibitor of MEK binds to a pocket adjacent to the ATP-binding site, which is referred to as the “allosteric back-pocket” (Ohren et al., 2004). These type 3 allosteric inhibitors can either bind in the presence or absence of ATP. Those that bind in the presence of ATP (DFG-in conformation, such as MEK inhibitors) can be referred to as “allosteric back-pocket DFG-in inhibitors”. Alternatively, “allosteric back-pocket DFG-out inhibitors” can bind to the allosteric back-pocket in the absence of ATP (DFG-out conformation) and include allosteric inhibitors of insulin-like growth factor 1 receptor (IGF-1R) (Heinrich et al., 2010), FAK (Tomita et al., 2013), or p38 (Over et al., 2013). In the case of IGF-1R, the inhibitor binds in the DFG-out pocket and extends over the catalytic loop rather than pushing it out from underneath like the FAK inhibitor, requiring additional conformational changes (Tomita et al., 2013). The allosteric AKT inhibitors are a special case of the allosteric back-pocket DFG-out inhibitors, as they only bind to this site when the pleckstrin homology domain is present (Barnett et al., 2005; Lindsley et al., 2005). Thus, the identification of this allosteric inhibitor that binds at the interface between the kinase domain and pleckstrin homology domain was only possible using the full-length protein for AKT (Barnett et al., 2005; Lindsley et al., 2005). While lack of competition with ATP has, in some cases, proven to be a useful way to identify type 3 inhibitors, it should be pointed out that the allosteric back-pocket DFG-out inhibitors will score as ATP competitive.

Type 3 inhibitors that are further away from the ATP site are the myristate (myr) pocket binders located in the bottom of the C-lobe of ABL (Adrian et al., 2006; Zhang et al., 2009; Fabbro et al., 2010), CHK1 inhibitors that occupy, in part, the substrate-binding site (Converso et al., 2009), and JNK1 inhibitors that occupy the mitogen-activated protein kinase insert region and A-loop (Comess et al., 2011), to only cite a few. A more comprehensive review on type 3 inhibitors has
been recently assembled by Cowan-Jacob et al. (Cowan-Jacob et al., 2014).

Rapamycins appear to be further removed from the kinase domain, as they specifically target the mTOR kinase in the context of the Raptor-containing mTORC1 complex (Wang and Sun, 2009; Yang et al., 2013). Similarly, agents targeting the extracellular domains of RTKs act outside the kinase domain (Christopoulos et al., 2014). The extracellular domains of RTKs can be targeted by peptide mimetics, peptoids, or antibodies (Fleishman et al., 2002; Udugama-sooriya et al., 2008; Cazorla et al., 2010; Jura et al., 2011). The monoclonal antibodies trastuzumab (Herceptin) and pertuzumab (2C4, Perjeta) act at different domains, with trastuzumab binding to domain IV and pertuzumab binding to subdomain II of the extracellular segments of the HER2/neu receptor, respectively (Cho et al., 2003; Hynes and Lane, 2005; Hsieh and Moasser, 2007). On the other hand, small molecules like SSR128129E [benzoic acid, 2-amino-5-[(1-methoxy-2-methyl-3-indolizinyl) carbonyl]-, sodium salt], which targets the extracellular D2D3 domains of the fibroblast growth factor receptor (FGFR), modulate signaling of the FGFR RTKs (Bono et al., 2013; Herbert et al., 2013). Examples of approved type 3 inhibitors are trametinib and the rapamycins.

Activators, Paradoxical Activation, and Priming. Targeting the allosteric sites on protein kinases may also lead to the identification of activators rather than inhibitors, which could be useful for therapeutic intervention or as pharmacological tools. In particular, compounds targeting the PIF pocket (the hydrophobic motif present in the N-terminal lobe of AGC kinases) of either PDK1 or PKCζ can either act as activators (Hindie et al., 2009) or substrate-selective inhibitors (Lopez-Garcia et al., 2011; Sadowsky et al., 2011; Busschots et al., 2012). Similarly, the myr-pocket binders of ABL can be converted into activators if they are designed not to bend helix I of the ABL kinase domain (Jahnke et al., 2010; Yang et al., 2011).

On the other hand, there are a few protein kinases that require activation rather than inhibition to fulfill their therapeutic task, like in the case of AMP-activated protein kinase and the insulin receptor for which activators have been identified (Li et al., 2001; Pender et al., 2002; Sanders et al., 2007; Lee et al., 2011; Salt and Palmer, 2012). PKC activation by exogenous compounds acting at the diacylglycerol-binding site can have tumor-promoting or tumor-suppressive effects (Martiny-Baron and Fabbro, 2007). These include phorbol esters, brostatin, and other compounds acting as diacylglycerol mimetics (Martiny-Baron and Fabbro, 2007). Another example of kinase activators includes a mimetic of the brain-derived neurotrophic factor that activates TrkB (NTRK-2) (Massa et al., 2010).

In some cases, kinase inhibitors can lead to unintended paradoxical activation either directly or via modulation of feedback loops. The most striking example is the paradoxical activation of RAF inhibitors, which can activate the mitogen-activated protein kinase pathway in certain genetic backgrounds (Hall-Jackson et al., 1999). This phenomenon is linked to a complex regulation of RAF due to crossactivation of the wild-type RAF isoforms, which is just beginning to be understood, almost a decade after the first so-called RAF inhibitor sorafenib was approved (Hall-Jackson et al., 1999; Hatzivassiliou et al., 2010; Poulilakos et al., 2010; Holderfield et al., 2013). Thus, BRAF inhibitors can activate MEK and extracellular signal-regulated kinase (ERK) signaling in normal cells, resulting not only in the absence of hypoproliferative toxicities but actual promotion of hyperproliferative toxicities (Su et al., 2012).

Another phenomenon is priming, which can lead to activation via kinase inhibitors, which has been observed for several kinases like AKT, MEK, and JAK (Okuzumi et al., 2009; Andruos et al., 2012; Hatzivassiliou et al., 2013). Priming describes the up-regulation of the phosphorylated form of the targeted kinase upon inhibition, which can lead to an activation of the pathway once the inhibitor is removed. Priming may also depend on the mode of action of the inhibitor. Inhibitors binding to active AKT do cause priming, whereas allosteric inhibitors targeting the inactive conformation of AKT do not (Lin et al., 2012).

There is currently no general strategy for the identification of allosteric kinase inhibitors or activators, as most of them have been discovered serendipitously by diverse approaches, ranging from phenotypic screenings to highly sophisticated structure-based drug design.

**Resistance to Kinase Inhibitors (in Oncology)**

**Introduction to Drug Resistance.** Most tumor cells express only a few of the 150 so-called “driver genes”, which, when mutated, promote tumorigenesis by affecting a dozen signaling pathways regulating cell fate, cell survival, and genome maintenance (Vogelstein et al., 2013). Insights into particular mutations and their consequences for signaling have already led to the development of more effective drugs (Siegel et al., 2012). The finding that deregulation of protein kinase activity by the gain of function mutations is essential to maintain the oncogenic state is also reflected by the fact that 30 kinase inhibitors have been approved for various oncological indications (Janne et al., 2009; Luo et al., 2009; Sharma and Settleman, 2010; Yarden and Pines, 2012) (Table 1). In many instances, these mutations can be linked to specific cancers (Greenman et al., 2007; Thomas et al., 2007). Although many activating mutations in various kinases have been found in a variety of cancers, it will take another effort to unambiguously identify the dependence of tumor growth on a particular kinase or its pathway(s). The major difficulty is the intrinsic heterogeneity of the late-stage forms of cancer that harbor multiple mutations, chromosomal aberrations, and display genomic instability. Demonstrating the cancer dependence of the kinase target will not only lead to the identification of inhibitors of the kinase, but may also accelerate the proof of concept in clinical trials, allowing a better selection of patients most likely to respond to targeted therapy.

Kinase inhibitors are being and have been designed to specifically target kinase alleles with a gain of functions (Blume-Jensen and Hunter, 2001; Fabbro and García-Echeverria, 2002a). Despite these successes, it should be emphasized that patients most likely to benefit from these kinase inhibitors often relapse after an initial response. Thus, the emergence of drug resistance is not limited to conventional chemotherapeutic drugs but extends to drugs with targeted modes of action (Engelman and Settleman, 2008a). The mechanisms of multidrug resistance to chemotherapeutic drugs have been studied and are not limited to reduced drug accumulation, but also involve changes in the level of target proteins, mutations that diminish drug binding, trapping of drugs in acidic vesicles, enhanced metabolism of drugs by cytochrome P450 mixed
function oxidases, increased tolerance of cellular DNA damage, and diminished apoptotic signaling (Gottesman, 2002; Szakacs et al., 2006; Hall et al., 2009). Apart from the usual mechanisms of drug inactivation in cancer (Szakacs et al., 2006; Hall et al., 2009) as well as the findings that quiescent tumor stem cells are refractory to kinase inhibitors (Graham et al., 2002), there are additional target-related mechanisms for resistance that are not based on mutations of the target kinase.

**Resistance to Kinase Inhibitors.** Drug resistance to targeted agents like kinase inhibitors can either occur by compensatory mechanism or by reducing the affinity of the kinase to its inhibitors (Szakacs et al., 2006; Fabbro et al., 2011).

The most commonly found point mutation leading to resistance concomitant with relapses affects the gatekeeper residue, whose size and shape regulates the properties of the hydrophobic pocket located at the back of the ATP-binding site. These mutations include the Thr gatekeeper of BCR-ABL1 (T315I) (Gorre et al., 2001; Sawyers, 2004; Fabbro et al., 2005), Kit (T670I) (Heinrich et al., 2003; Fletcher and Rubin, 2007), PDGFRα (T674I) (Cools et al., 2003), PDGFRβ (T681I) (Daub et al., 2004), SRC T341M (Bishop, 2004), as well as other types of gatekeepers like L1196M in ALK (Katayama et al., 2012), G697R in FLT3 (Cools et al., 2004), and V561M in FGFR1 (Blencoe et al., 2004). This is either due to a steric clash between the inhibitor and mutated gatekeeper (Blencoe et al., 2004; Fabbro et al., 2005; Fabbro et al., 2011) or by significantly increasing the affinity for ATP and thereby reducing the affinity for the kinase inhibitors (Kobayashi et al., 2004; Pao et al., 2005). Mutations in the gatekeeper as well as other kinase domain mutations confer resistance to a wide spectrum of kinase inhibitors without affecting the kinase activity and may explain a fraction of cases of acquired resistance.

There are additional mechanisms to circumvent kinase inhibition (Sawyers, 2004; Rubin and Duensing, 2006, Fabbro et al., 2011; Serra et al., 2011; Logue and Morrison, 2012; Trusolino and Berlotti, 2012; Workman et al., 2013). Compensatory changes in the signaling pathways of treated cancer cells that can bypass drug-mediated kinase inhibition and so restore downstream signaling in the presence of drug include

1. Amplification of the target, like in the case of BCR-ABL1 in CML (le Coutre et al., 2000) or dimerization of aberrantly spliced BRAF (V600E) (Poulilakos et al., 2011); and
2. Up-regulation of RTKs following inhibition of PI3K (Serra et al., 2011; Rodon et al., 2013) or up-regulation of alternative kinase pathways through the receptors for hepatocyte growth factor (MET), IGF-1R, or AXL in the acquisition of resistance to EGFR kinase inhibition (Engelman et al., 2007; Turke et al., 2010; Logue and Morrison, 2012). Activation downstream of RTKs, like the RAS-RAF-ERK and/or PI3K/AKT pathways, by several mechanisms can override the effects of any RTK inhibitor. Examples include activation of the PI3K/AKT pathway by activating point mutations in PI3K subunits, loss-of-function/deletions of the PTEN phosphatase in EGFR-mutated lung cancer, mutations in RAS isoforms downstream of oncopgenic RTKs, MEK activation by COT1 bypassing inhibited BRAF-V600E, and activation or the EGFR-mediating resistance to vemurafenib in BRAF-V600E (She et al., 2003; Johannessen et al., 2010; Prahallad et al., 2012). Signaling redundancies, interconnections through pathway crosstalk, and negative feedback loops have also been identified as contributors to drug resistance or weaken the efficacy of kinase inhibitors (Janne et al., 2009; Rodrik-Outmezguine et al., 2011; Chandarlapaty, 2012). Negative feedback loops, crosstalk, crossactivation, and pathway convergence connect signaling pathways through activated components and are crucial for the maintenance of normal cell functions as well as the dynamic and adaptive responses to extracellular signals (Mendoza et al., 2011; Logue and Morrison, 2012). Even if a driver kinase is suppressed by the inhibitor, the tumor cells can exploit its interconnected signaling pathways to evolve drug resistance (Trusolino and Berlotti, 2012). The Ras-ERK and PI3K-mTOR signaling provide major mechanisms for the regulation of cell survival, differentiation, proliferation, metabolism, and motility in response to extracellular ligands and are activated in over 80% of all tumors (Engelman, 2009; Wong et al., 2010). Selective inhibition of mTORC1 leads to disruption of a negative feedback loop, which enhances the activity of PI3K and its effector AKT, counteracting the antiproliferative effects of mTOR inhibition (Chandarlapaty, 2012). Combined inhibition of the mTOR kinase and RTKs therefore seems a promising approach to abolish AKT signaling and prevent resistance formation (Rodrik-Outmezguine et al., 2011). Alternatively, inhibition of PI3K/mTOR signaling resulted in the activation of the Jak/STAT5 pathway, suggesting a combination strategy targeting both the PI3K/mTOR and JAKSTAT5 signaling (Britschgi et al., 2012). Negative feedback regulation has also been observed in the RAS-RAF-ERK cascade (Mendoza et al., 2011). The binding of vemurafenib to wild-type BRAF in cells can cause BRAF/CRAF heterodimerization and paradoxical activation of ERK1/2, which can result in the development of kerato-acanthomas in patients (Chapman et al., 2011; Poulilakos et al., 2011; Su et al., 2012; Holderfield et al., 2014). This is further exacerbated in the case where oncogenic RAS and BRAF mutants are present in the same cells that support RAS-dependent dimerization and paradoxical ERK activation.

3. Factors regulating the bioavailability and intracellular concentration of inhibitors like poor intestinal absorption, tight binding to blood plasma proteins, overexpression of the multidrug resistance genes, and/or increased metabolism of the drug by liver cytochrome P450 proteins have also been linked to primary resistance (Mahon et al., 2003; Apperley, 2007).

All of these mechanisms demonstrate the plasticity of cancer cells and the many ways by which a tumor can evade targeted therapies. The only way to approach these problems is to use a rational combination of drugs, which is a mainstay in cancer therapy.

**Breaking the Resistance to Kinase Inhibitors.** To circumvent target-dependent drug resistance, second-generation kinase inhibitors have been developed. Inhibitors that bind covalently to the ATP-binding site of EGFR have been developed for the emerging resistance to gefitinib and erlotinib (Kwak et al., 2005; Heymach et al., 2006; Felip et al., 2007;
Several kinase inhibitors have become successful drugs. A large number of kinase inhibitors are in clinical development mainly for oncology indications, which is a testimony for the greater tolerability in this indication with respect to potential side effects. One of the issues that is perceived as a major challenge in kinase drug discovery is the selectivity for the target kinases, which defines the on- and off-target pharmacology (Cohen, 2002; Fabbro and García-Echeverría, 2002a,b; Vieth et al., 2004, 2005; Cowan-Jacob, 2006; Liu and Gray, 2006; Zhang et al., 2009). While development of selective kinase inhibitors is likely to be extremely important from the standpoint of minimizing drug side effects, inhibitors with exquisite selectivity are a must for chronic administration in non-life-threatening diseases like many immunologic dysfunctions (Cohen, 2002; Fabbro and García-Echeverría, 2002a; Fabbro et al., 2002b; Vieth et al., 2004, 2005; Liu and Gray, 2006; Zhang et al., 2009). While the lack of selectivity of kinase inhibitors can be advantageous in cancer treatment, it can also lead to side effects (Cheng and Force, 2010). On the other hand, low selectivity complicates mechanism of action studies as well as biomarker discovery. The information linking adverse side effects of protein and lipid kinase inhibitors with a particular protein kinase selectivity profile is only now beginning to emerge (Force et al., 2007; Olaharski et al., 2009). Moreover, we are still trying to understand the side effects that are generated by inhibiting multiple kinases, including undesired kinases that may generate, at least in part, the adverse side effects. Recent data, for example, suggest that simultaneous inhibition of ATM, ATR, DNA-PK, Aurora, and/or other kinases involved in mitosis may be genotoxic (Force et al., 2007; Olaharski et al., 2009).

The development of kinase inhibitors for non-life-threatening indications where chronic regimens are being used will require a better target selectivity profile to minimize side effects. Therefore, only few attempts have been made to position kinase inhibitors in chronic diseases, such as inflammation and immune disorders. Most prominent is the p38 kinase, which has been targeted for treatment of nononcology indications like rheumatoid arthritis.

Addressing the noncatalytic functions of kinases will result in novel types of kinase inhibitors with improved selectivity. The design and synthesis of non-ATP competitive (allosteric) kinase inhibitors with high selectivity will not only lead to the expansion of the kinase target space with less studied members of the family like the atypical and the pseudo-kinases. Having highly selective kinase inhibitors (ideally against most of the members of the whole kinase) at hand will propel the pharmacology as well as understanding of their roles in signaling and regulation. Thanks to the available kinase inhibitors, we have learned a lot about drug-protein interactions and cancer cell signaling, in some cases only after their use in the clinic. We only now begin to appreciate the complex rules determining the mechanism of action and specificity of these types of inhibitors. In addition to its clinical use, the various new drugs and drug candidates will be instrumental and indispensable reagents for many biologic disciplines to help decipher kinase regulation, interrogate signaling pathways, perturb cellular networks, and study complex cell biologic processes.

One of the major challenges in future kinase drug discovery is to better understand the cancer dependence of the target kinase and anticipate the emerging resistance to kinase inhibitor treatment. With a few exceptions, the development of kinase inhibitor resistance in cancer has lowered the enthusiasm about the initially observed outstanding clinical
effects. Although, in some cases, we have seen long-lasting clinical responses with these kinase inhibitors, we have inevitably and not unexpectedly seen relapses under treatment. Understanding resistance mechanisms has not only lead to a deeper understanding of cancer cell biology, but also instructed ways to overcome resistance using second-generation kinase inhibitors with a better selectivity and appropriate potency, which are being applied to a genetically better defined patient population that is most likely to respond. In addition, clever combinations and sequential treatment regimens are being tested. It should be noted that clinical responses have also been noted with many other kinase inhibitors without a clear rationale, offering encouragement that single agents can be active in genetically complex neoplasms, although it is formally possible that the single-agent activity may be due to its fortuitous ability to inhibit multiple kinases. The recent introduction of the concept of individualized cancer therapy along with the development of selective drugs targeting specific alteration in tumors has provided hope for the development of more effective treatment strategies.

In summary, the available complement of clinically used kinase inhibitors do not cover more than 10–15% of the whole kinase. Ongoing efforts using genome-wide screening in conjunction with the use of genetic organisms will unravel new disease associations and pave the way for the discovery of many more new protein kinase targets in the coming years.

Finally, there are many selective protein kinase inhibitors that cannot be used as drugs for reasons of toxicity or solubility but which are still extremely useful as research reagents (Robert et al., 2005; Force et al., 2007). Extending the complement of very selective (allosteric) protein kinase inhibitors in conjunction with a system biology approach will advance our understanding of kinase biology in cellular networking and diseases.

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Fabbro


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