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Perspective

Kinase Domain Mutations in Cancer: Implications for Small Molecule Drug Design Strategies

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1. Introduction to Kinases and Kinase Inhibition

Kinases have emerged as ubiquitous but highly challenging targets for drug discovery. The human genome has 518 kinases[1] and many of these play critical roles in cell growth and apoptosis, making them interesting drug targets for oncology. Kinase targets including epidermal growth factor receptor (EGFR[a]), Raf, Src, and breakpoint cluster region—Abelson's kinase (bcr-Abl) emerged early in the study of oncogenic proteins.[2] Despite years of effort involving a compelling breadth of accumulated preclinical design experience (reviewed extensively by Liao and Andrews[3,4]) and clinical experience (reviewed by LaRusso and Eder [2]), relatively few kinase inhibitors have been approved (Table 1). As an additional complication, clinical use of these inhibitors has led to the emergence of drug resistant tumors.[5−8] In many patients, response to small molecule kinase inhibitors has been followed by tumor resurgence, which rendered these inhibitors less effective than expected. This resistance has been linked to a number of mechanisms that include the amplification of the oncogenic kinase gene[9] and alternative signaling pathways or plasticity in signaling.[10] However, in many instances, resistance has been traced to individual or groups of mutations in the drug targets that make the tumors unresponsive in the clinic. These mutants alter the binding properties of the drugs as shown by in vitro studies.[5] When viewed across multiple cancer targets, the location of these mutations forms a compelling pattern with a number of common mechanisms elucidated with reference to this pattern. Significantly, recent characterization of mutations in the EGFR kinase has included structural and kinetic studies that have challenged assumptions about how individual drug resistance mutants are understood and to what extent mechanisms can be generalized across kinases by homology alone. This review will provide a brief overview of kinase structure and function as it pertains to drug discovery, describe the location and importance of clinical mutations, and review the emerging understanding of their impact based on sequence homology, protein crystal complexes, and biochemical/biophysical information. Underlying this discussion is our appreciation that the current clinical arsenal of small molecule kinase inhibitors only contains the first weapons to be deployed in a long war against drug resistance mutations occurring in multiple kinases that target multiple cancers.

Table 1 provides a list of kinase inhibitors approved to date for various cancer indications within the U.S. Since 2001, eight inhibitors targeted to the kinase catalytic domain have been approved for clinical use led by imatinib mesylate for chronic myeloid leukemia (CML).[11,12] CML has been traced to the effect of a characteristic mutation in which part of the breakpoint cluster region (BCR) gene was spliced into the Abelson kinase (Abl) gene creating a hybrid BCR-Abl gene. Known as the "Philadelphia chromosome", the splice occurs upstream of the kinase domain. Nevertheless, pathway inhibition, by imatinib treatment (Figure 1), provides a significant benefit to patients until the emergence of resistant tumor strains. Gene sequencing efforts on the resulting resistant tumors identified a number of mutations, many in the kinase domain, which reduced the

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[a] Abbreviations: Abl, Abelson kinase; AML, Acute Myeloid Leukemia; AMP-PNP, 5′-adenylyl-β,γ-imidodiphosphate; ATP, adenosine triphosphate; Bcr-Abl, breakpoint cluster region—Abelson kinase; EGF(R), epidermal growth factor (receptor); CML, chronic myeloid leukemia; CMML, chronic myelomonocytic leukemia; CMPD, chronic myeloproliferative disorders; DFSP, dermatofibrossarcoma protruberans; Erb-B1/2/3/4, erythroblastic leukemia viral oncogene homologue 1/2/3/4; Erb-B1 is synonymous with EGFR and HER-1; GIST, gastroInstestinal stromal tumor; HER-1/2/3/4, human EGF receptor-1/2/3/4; HES, hypereosinophilic syndrome; NSCLC, non-small-cell lung cancer; PDGF(R), platelet derived growth factor (receptor); PKA, protein kinase A; SMCD, systemic mast cell disease; VEGF(R), vascular endothelial growth factor (receptor); WT, wild type.

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Table 1. Kinase Inhibitors Approved for Use in the U.S. as of August 2008

U.S. brand name	year approved	generic name	U.S. FDA-approved indications	company	target kinases
Gleevec	2001	imatinib mesylate	chronic myeloid leukemia (CML)	Novartis	Abl, c-Kit, PDGFRa, PDGFRb
Iressa	2003	gefitinib	oncology, non-small-cell lung cancers (NSCLC)	AstraZeneca	EGFR
Tarceva	2004	erlotinib	NSCLC, pancreatic cancers	Genentech, OSIP	EGFR
Nexavar	2005	sorafenib tosylate	hepatocellular carcinoma, renal cell carcinoma	Bayer and Onyx	Raf, VEGFR2, VEGFR3, c-Kit, PDFGRb
Sutent	2006	sunitinib malate	gastrointestinal stromal tumor (GIST), renal cell carcinoma	Pfizer	c-Kit, VEGFR, PDGFR, FLT3
Sprycel	2006	dasatinib	CML (especially imatinib-resistant)	Bristol-Myers Squibb	Abl, c-Kit, PDGFR, Src
Tasigna	2007	nilotinib	CML (imatinib resistant and intolerant)	Novartis	Abl, c-Kit, PDGFRb, Src, Ephthrin
Tykerb	2007	lapatinib	breast cancer	GlaxoSmithKline	EGFR, Her-2

effectiveness of imatinib. Two additional drugs, nilotinib and dasatinib, have since been approved specifically for imatinib-resistant or unresponsive tumors in CML. The history of the development and spectrum of the activity of current inhibitors, including those now in clinical trials, have been the subject of several recent reviews.[11,13−15]

The second and third kinase inhibitors to be approved for clinical use were gefitinib and erlotinib, two inhibitors of EGFR kinase (Figure 1). These drugs worked remarkably well in a subpopulation of patients with non-small-cell lung cancer (NSCLC) harboring activating mutations.[7,16−22] Unfortunately, drug resistant tumors often emerged within a year or so of initiating treatment. In 2007, a third inhibitor, lapatinib (Figure 1), was approved for use in breast cancer in combination with a cytotoxic agent; lapatinib is a dual inhibitor of both EGFR and human EGF receptor-2(HER-2) kinases.

The other class of clinically approved inhibitors targets multiple kinases associated with angiogenesis and has a broader spectrum of inhibition against such tyrosine kinases (Figure 1). Sorafenib inhibits multiple vascular endothelial growth factor (VEGF) receptor kinases, platelet derived growth factor (PDGF) receptor kinases, the mast-stem cell growth factor receptor (c-KIT) kinase, and the proto-oncogene c-Raf kinase at clinically relevant concentrations. Sunitinib inhibits VEGF and PDGF receptor kinases, as well as c-Kit and FL tyrosine kinase 3 (Flt3). These drugs are expected to be effective by arresting the development of blood supplies to the growing tumors, in

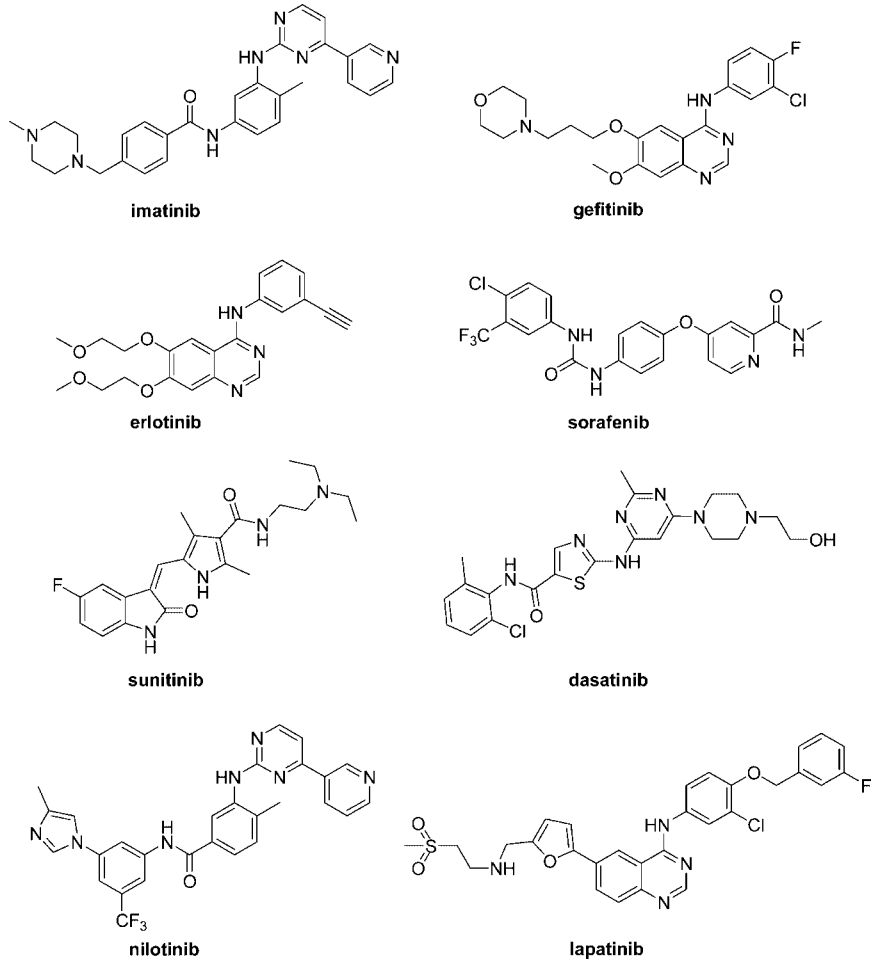

Figure 1. Structures of kinase inhibitors marketed in the U.S. as of August 2008.

addition to specifically blocking an oncogenic kinase within a tumor type (e.g., c-Kit).[23−25]

The story of the development of these kinase inhibitors is partly the story of the discovery and progressive exploitation of multiple conformational states of the kinases. Early on, selectivity was identified as a challenge when designing inhibitors that bound at the adenosine triphosphate (ATP) pocket. Since ATP is a cofactor that is essential to kinase function, evolutionary pressure has been exerted to maintain a general common shape and chemical similarity to its binding site in different kinases. Because of this, drug design efforts have sought to exploit regions of the active site that are not directly involved in ATP binding, or conformations of the kinase that show greater structural and chemical heterogeneity. The opportunity to exploit conformational heterogeneity is a consequence of the activation and inactivation mechanisms of kinases. As the inactive form of the kinase does not have to obey the requirement of binding to the common substrate ATP, greater structural variation has been observed in the inactive states of kinases. Notably, imatinib, the first kinase inhibitor to make it to market, binds to an inactive form of the Abl, c-Kit, and PDGF kinases.[14]

The clinical experiences with the kinase inhibitors, particularly in terms of patient response and resistance to drugs, have stimulated vigorous efforts in search of solutions that will aid in the design of the next generation of inhibitors. Resistance to inhibitors is expected to occur because of several mechanisms including gene amplification[9,10] or mutations in the kinase domain, which include specific mutations in amino acid residues or deletions of certain sequences. From a technical drug design perspective, this aspect represents a remarkable opportunity to design compounds with enhanced biological properties, but its complexity from a practical point of view is substantial. Underlying this complexity are the effects of mutations in the kinase domain on ATP affinity, catalytic activity, and (global) dynamic effects on kinase structures (active vs inactive forms) in addition to effects on the binding site character, (local) movement of amino acid residues, and nature of key interactions with the protein. Chemists have to integrate multidimensional information from various sources to develop design strategies for the next generation of drugs having enhanced properties over current compounds.

Kinase Activation Relevant to the Design of Clinical Inhibitors: DFG Motif Dynamics. Kinases control and amplify intracellular signals by the selective phosphorylation of residues on other proteins, often other kinases, or even the same kinase. Upon activation, the kinase binds ATP and the terminal phosphate group of ATP is abstracted and subsequently transferred to the substrate. Kinases share a characteristic ATP-binding structure shown in Figure 2. The kinase or catalytic domain consists of an N-terminal lobe, which consists mainly of β strands but contains one α helix, helix C. The C-terminal lobe is mainly α-helical in nature, and a short strand termed the hinge region connects the two lobes. The ATP binding site is sandwiched between the lobes where ATP forms critical hydrogen bonding interactions to the hinge region. The P-loop (or phosphate-binding loop or glycine-rich loop), with the sequence of G-X-G-X-X-G, plays a large role in the dynamics of the kinase domain where its conformation is a determining factor in the shape of the ATP-binding site (Figure 2). In the active kinase, a characteristic Asp-Phe-Gly motif (DFG motif), which is located immediately before the activation loop, adopts a conformation with the Asp and Phe both oriented toward the binding site (DFG-in) (Figures 2 and 3a).

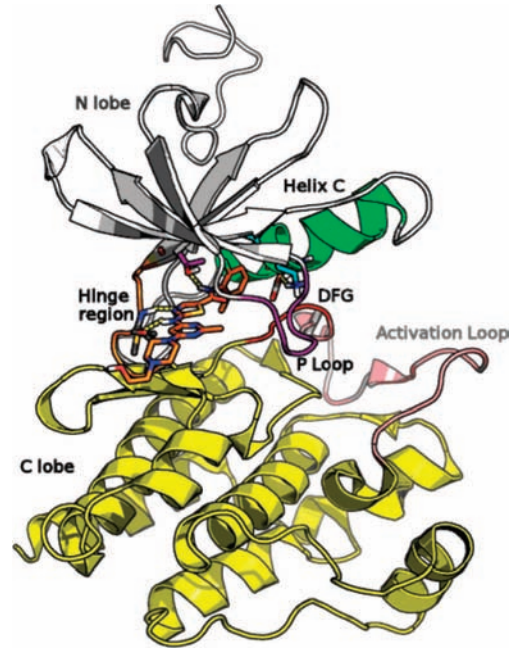

Figure 2. Crystal structure of Abl in the active form bound to dasatinib (2GQG.pdb) in ribbon depiction. The top part, mostly in white, is the N-terminal lobe. The C-terminal lobe at the bottom is shown in yellow. The hinge region connecting the two lobes is colored orange, and dashed yellow lines show the hydrogen-bonding interactions of dasatinib to the hinge region. Also shown is the hydrogen bond of the inhibitor to the gatekeeper residue Thr315 (magenta carbons). Helix C at the back of the binding site is colored green, and the P loop is shown in purple. The activation loop (pink) and the DFG motif (red) are fully resolved in this structure. The catalytic lysine (cyan carbons) is in proximity to the glutamic acid (white carbons) from helix C, allowing the formation of a salt bridge, as expected in the active form.

Whereas the active form of the kinase catalytic domain is fairly homogeneous structurally, determined by the requirement to be able to bind ATP, the inactive forms are less so. Different inactive states have been identified, which can be related to the mechanism of activation of the kinase.[26] One of these states is often called the "DFG-out" state and is associated with kinases that activate by phosphorylation of residues on the activation loop. In the DFG-out state, the phenylalanine of the DFG motif is positioned in the ATP-binding site so that it effectively blocks ATP access to its binding site (Figure 3b). Phosphorylation of tyrosine, threonine, or serine residues of the activation loop by another kinase is incompatible with this conformation and subsequently leads to activation. Phosphorylation can occur because the activation loop can act as a substrate to other kinases or can even be phosphorylated autocatalytically.

Crystal structures of kinases in the DFG-out conformation have been solved for Abl, c-Kit, IRK, Flt3, CSK, Raf, and P38.[27] This state is a key target of drug design for these kinases. Kinase inhibitors that bind to the active form of the kinase are often called type I inhibitors, and those that bind to the inactive form of the kinase are called type II inhibitors.[27] According to this classification, dasatinib is a type I inhibitor and imatinib and nilotinib are clinical type II inhibitors.

In the past year, several NMR studies of kinase catalytic domains have been reported in the literature. Studies of protein kinase A (PKA) with addition of the nonhydrolyzable ATP analogue AMP-PNP and a peptide substrate highlighted the areas that undergo conformational changes upon binding of ATP and substrate. Interestingly, it was shown that the DFG region accesses multiple conformational states, presumably "flipping"

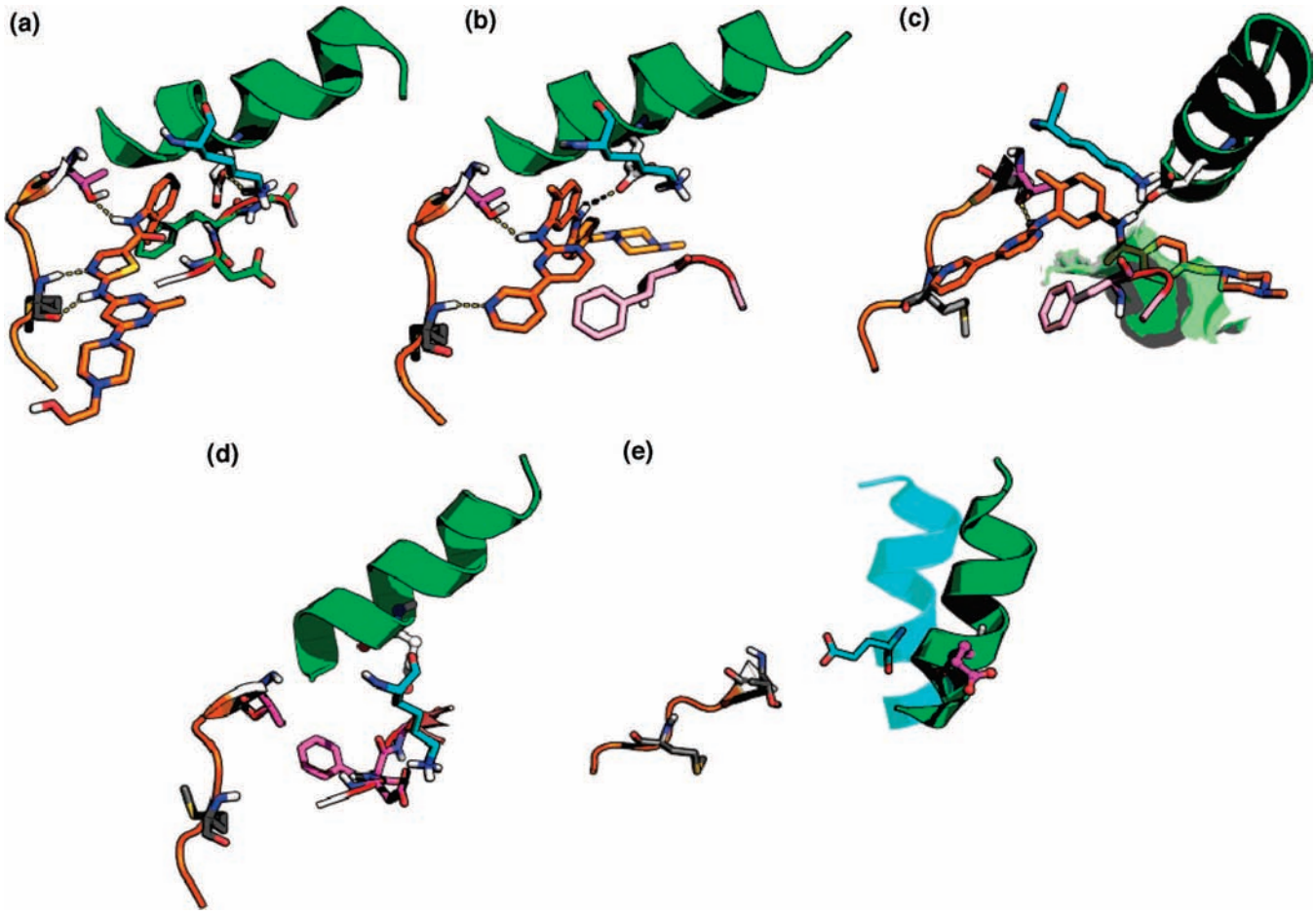

Figure 3. Binding site comparisons of active and inactive forms of different kinases. (a) The Abl active form in complex with dasatinib (2GQG.pdb). The phenylalanine of the DFG motif (green carbons) is shown to point away from the binding site and is buried in the interior of the protein. (b) The Abl DFG-out inactive form in complex with imatinib (1IEP.pdb). The phenylalanine of the DFG motif is shown in pink carbons and points into the binding pocket. (c) Rotation of ~90 degrees of (b) to highlight the depth of the DFG-out pocket (surface representation). (d) Helix-C-out inactive form of EGFR (1XKK.pdb). The catalytic lysine (cyan carbons) is far removed from Helix C's glutamic acid (white carbons). (e) Helix-C movement from active (cyan helix) to inactive form (green helix) from an overlay of the Abl (2GQG.pdb, helix in) and EGFR (1XKK.pdb, helix out). (a)−(c) show the catalytic lysine (cyan carbons) and the salt bridge formed with the glutamic acid from Helix C (white carbons).

from DFG-in to DFG-out, in the presence of AMP-PNP and with both AMP-PNP and peptide substrate bound. X-ray crystallography studies of PKA showed a single conformation for this region.[28] NMR studies of Abl in complex with type I and type II inhibitors highlighted conformational differences in the DFG region and the activation loop in complex with these different types of inhibitors. The mechanism of inhibition of imatinib and nilotinib (type II) and dasatinib (type I) could be assigned unambiguously on the basis of the NMR data.[29] In contrast to the PKA NMR studies highlighted above,[28] the DFG region was fully resolved in all three Abl complexes and thus resides in a single conformation.[29] The Abl NMR studies with PD-180970[30,31] (**1**, Figure 6), a type I inhibitor, highlight that X-ray crystallography can trap protein conformations that are not highly populated in solution.[29]

Kinase Activation Relevant to the Design of Clinical Inhibitors: C-Helix Dynamics. A second inactive form of the kinase, most commonly associated with EGFR, retains the general DFG-in form but leads to inactivation by rotating and shifting the C-helix out (Figure 3d−e).[32,33] Different activation mechanisms have been associated with the helix-C-out inactive conformation. In c-Src, activation is effected by phosphorylation of a tyrosine in the activation loop, in much the same way as phosphorylation of Abl destabilized the DFG-out inactive form. In CDK2, binding of the protein cyclin to the kinase appears to be the key event effecting activation. In EGFR, the formation of an asymmetric kinase domain homo- or heterodimer upon the extracellular binding of EGF leads to kinase activation.[34,35] Although EGFR family kinases have a tyrosine in the activation loop that gets phosphorylated, this does not appear to be necessary for activation.[34] The C-helix-out form of the kinase has been shown to bind lapatinib[32] and the clinical candidate **8** (neratinib, HKI-272, Figure 8).[33]

The C-helix-out inactive form can alter the nature of the ATP binding site. Upon rotation of helix C in EGFR, a conserved glutamic acid points toward solvent rather than toward the ATP binding site (Figure 3d). This conformation disrupts a salt bridge between the glutamic acid and the conserved catalytic lysine residue. This salt bridge is key in positioning the α and β phosphate groups of ATP and is thus critical for catalytic activity. The C-helix motion also appears to draw the P-loop down to further close the ATP binding cleft (compare Figures 9 and 10). Key structural changes associated with the movement from C-helix-out inactive form to the active form are shown in Figure 3d and Figure 3e. Crystal structures of EGFR bound to ATP analogues in the DFG-in/C-helix-out conformation have been solved for EGFR.[34] This opens the possibility that this state may be ATP-bound under normal conditions.

A number of recent studies have suggested that these inactive states may not be mutually exclusive. A C-helix-out conforma-

Figure 4. Kinase domain mutations identified in EGFR, ABL, and PDGF and c-Kit. Mutants that activate the kinase or cause resistance by activating the kinase are shown in magenta. Mutants that lead to resistance either directly or in conjunction with an activating mutant are shown in bold blue. Although not directly linked to resistance mutations, activating mutations of b-Raf are also shown emphasizing the patterns arising in the resistance mutants.[114,115]

tion of an inhibitor bound to Abl has been solved.[36] Conversely, a crystal structure of c-Src bound to a close analogue of imatinib in a DFG-out inactive conformation has also been solved.[37] The authors of this latter study, however, note that achieving this conformation appears to come at a substantial thermodynamic penalty, reflected in inhibitor potency, that cannot be attributed to the effect of individual amino acid changes. Overall, these observations provide insight into the dynamics of kinases. The value of different structural forms from a drug design perspective is unknown, as the thermodynamic penalties associated with these atypical states could be substantial and might therefore not be effective targets for inhibition.

Emergence of Drug Resistance. The emergence of drug resistant tumors was an unwelcome addition to the already substantial challenges to kinase drug discovery. Kinases were expected to be difficult targets partly because the conservation of shape and character of the ATP binding site posed a selectivity issue and partly because the high endogenous concentration of ATP suggested that inhibitors would have to be very potent to succeed clinically. Notably, after the first kinase inhibitors were used clinically, it became clear that various amino acid mutations, some in the kinase domain, could lead to drug resistance.[5,18,19,38] Since then, overcoming drug resistance has become a defining challenge of kinase drug discovery to existing targets. Figure 4 shows an alignment of parts of the sequences of kinases that are relevant to the action of kinase inhibitors in clinical use and onto which the location of key mutations have been mapped. Repeating patterns of

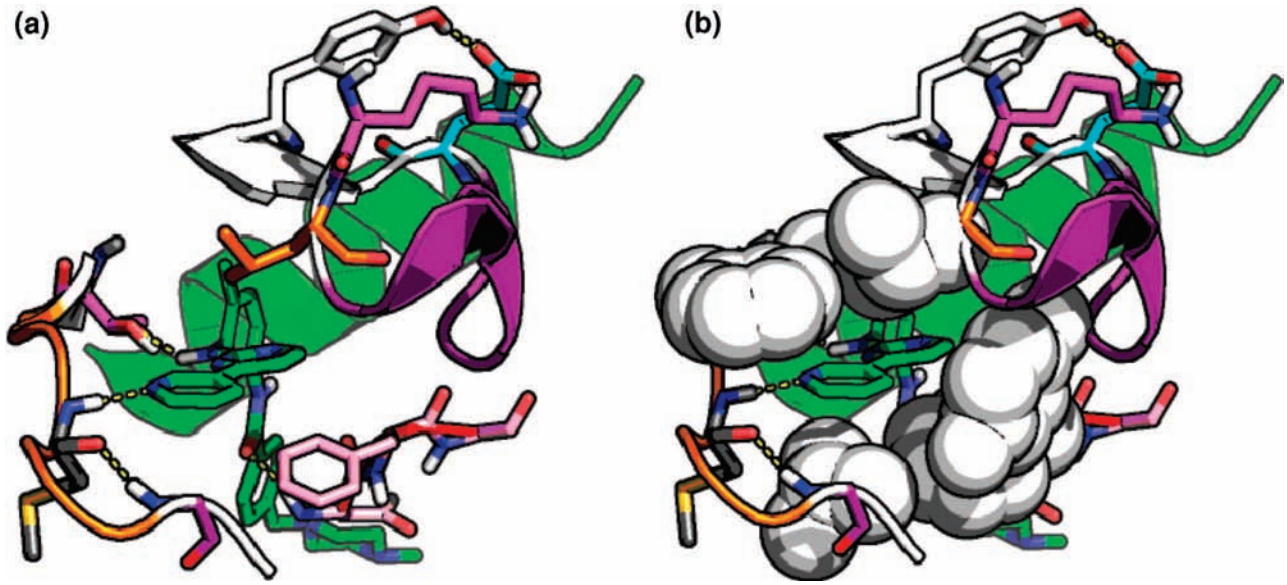

Figure 5. (a) Close-up of binding site of Abl bound to imatinib (1IEP.pdb). Hydrogen bonding interactions of imatinib to the hinge region (Met 318 in gray carbons) and T315 (magenta carbons) are indicated. Several drug-resistant mutation sites are shown. Gly321, in the front of the binding, is shown in purple carbons, and the P-loop residue L248 is shown in orange carbons. Also shown is Glu255 (cyan carbons) and its proximity to Lys247 (magenta carbons) and Tyr 257 (white carbons). (b) Close-up of the hydrophobic enclosure of imatinib (1IEP.pdb). The hinge-region hydrogen bond to Met 318 is shown.

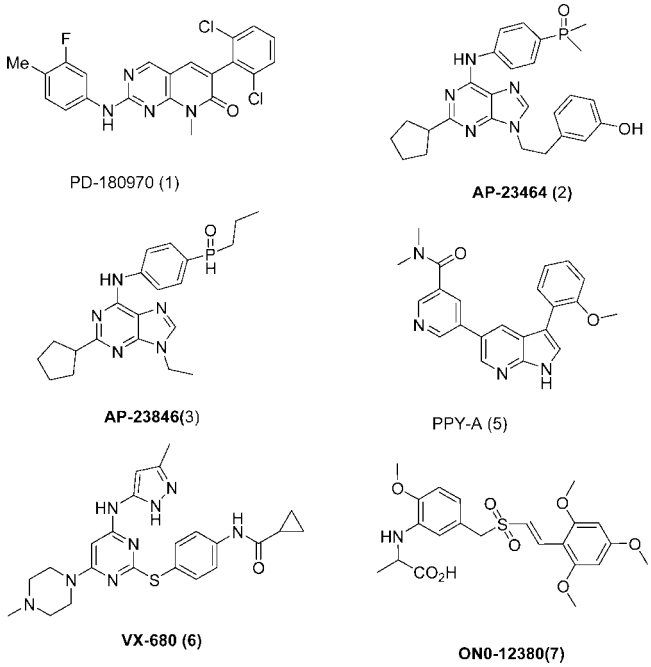

Figure 6. Preclinical and clinical phase inhibitors of bcr-Abl.

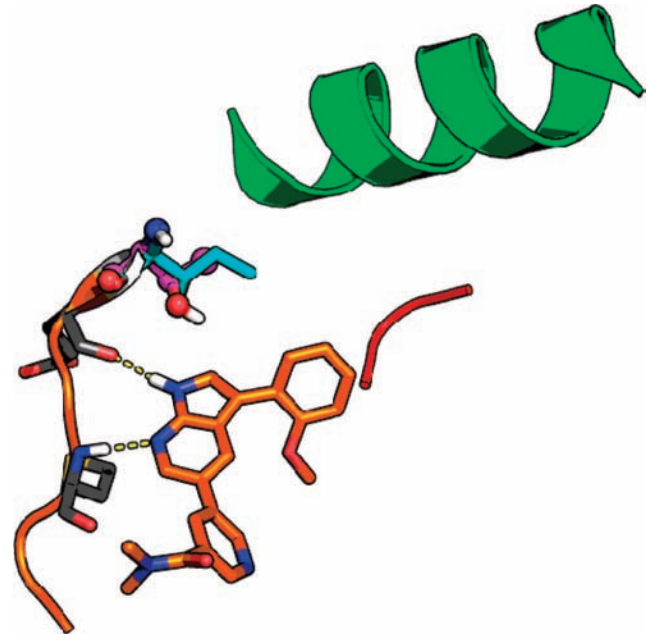

Figure 7. Crystal structure of an analogue of **4** in complex with Abl, highlighting its distance from the gatekeeper residue T315 (magenta carbons, 2QOH.pdb) and the Ile 315 mutant (cyan carbons, 2Z60.pdb).

mutations appear at the activation loop, at the P-loop, and at the "gatekeeper" residue within the ATP binding sites. Individual kinases have additional mutations that appear at unique locations, most of which can result in alterations of ligand binding or kinase activation. The emergence of crystallographic data for complexes of key protein−ligand pairs with mutations in Abl[39−41] and EGFR[33,42] has helped interpretation of the consequences of these mutants.

Some general trends are evident. Mutations in the P-loop and activation loop destabilize inactive forms of the kinases in favor of active states. In this way, they reduce or eliminate binding of inhibitors targeted at the inactive forms of the kinases. This mechanism of drug resistance is equivalent to the effect of oncogenic activating mutations (cf. c-Kit[43] and PDGFR [44,45]). The effects of a number of mutations can also be rationalized by their direct action on ligand binding within the ATP-binding cleft or occupation of additional accessible space in the inactive form of the kinase. Most notable of this type of mutation is that of the gatekeeper threonine in Bcr-Abl and c-Kit, which confer resistance to imatinib. This has been rationalized as a direct loss of a critical hydrogen bond to the ligand (Figure 3b). An intriguing exception to this pattern occurs in EGFR kinase, in which mutation of the gatekeeper residue appears to restore ATP affinity lost because of co-occurring activating mutations.[33]

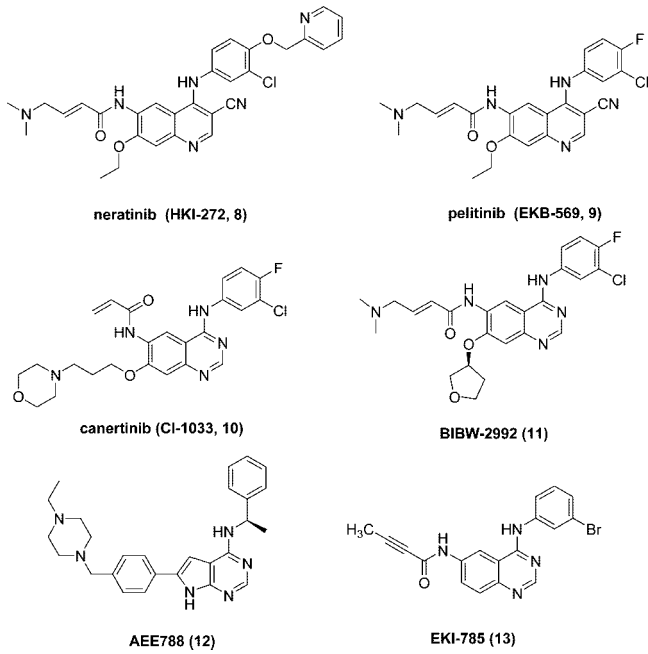

Figure 8. Preclinical and clinical investigational inhibitors of EGFR kinase.

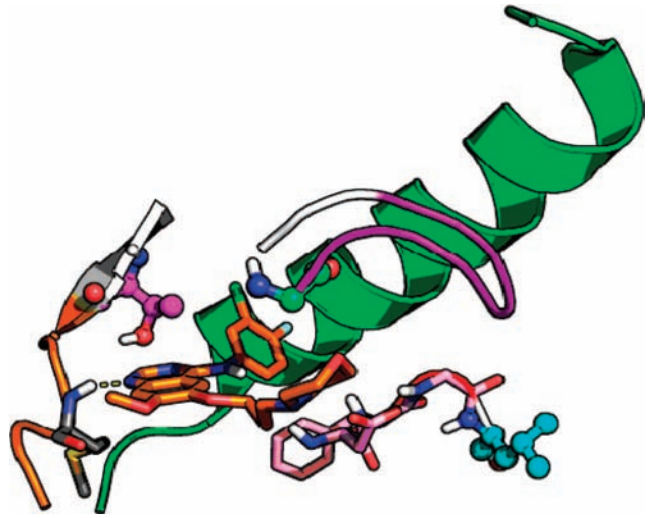

Figure 9. Protein crystallographic complex of EGFR in the active form with gefitinib (2ITY.pdb). The central ring interacts with Met 793 of the hinge (gray carbons) with a single hydrogen bond. The DFG motif is shown in pink carbons. The locations of key mutant residues are shown. Glycine 719 (green carbons) lies in the P-loop, and Thr 790 (gatekeeper, magenta carbons) forms part of the binding pocket. Leu 858, which is part of the activation loop (cyan carbons), does not interact with the inhibitor.

2. Protein Structural Interpretation of Drug Resistant Mutations

How much do we now understand about the key mutations in various kinases? In this section, the key resistance mutations of the kinase targets relevant to the current clinical drugs will be described. Where possible, the effect of the mutation on ligand binding will be discussed on the basis of crystallography and, if available, biochemical studies. Important clinical candidates that appear to overcome known resistant mutants will also be identified. Together, this information points toward strategies that might overcome known resistant mutants and anticipated resistance mutations that could emerge for new inhibitors and targets as a consequence of clinical use.

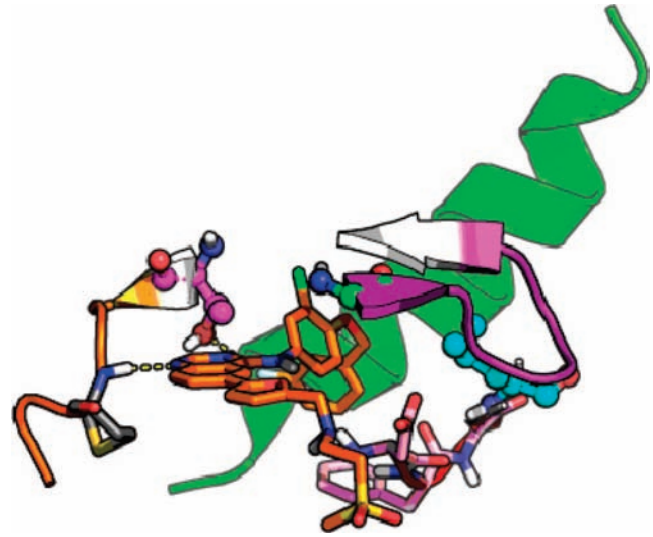

Figure 10. Lapatinib bound to the C-helix-out inactive form of EGFR kinase (1XKK.pdb). Threonine 790 (magenta carbons) makes a favorable interaction with the fluorophenyl group of the inhibitor. The DFG motif is shown in pink carbons. Glycine 719 (green carbons) lies in the P-loop and forms part of the binding cleft. The significant change in conformation of the DFG motif (compare to Figure 9) causes Leu 858 (cyan carbons) to be directed toward the binding region.

BCR-Abl. The approval of imatinib (Gleevec, STI-571) in 2001 was a major breakthrough not only in the treatment of CML but also for cancer therapeutics in general. Imatinib was the first targeted cancer therapy to enter the clinic that specifically inhibited the kinase domain of the BCR-Abl oncogene. Despite the initial success of imatinib treatment, most patients will eventually develop resistance. Dozens of clinical mutations in the kinase domain of this enzyme have been described.[46−49] The most commonly observed mutations are T315I, E255K, and M351T, which account for more than 60% of the cases (Table 2).[49−51]

Imatinib was shown by X-ray crystallography to inhibit Abl kinase by binding to the DFG-out form of the kinase domain.[52] The observed relapse of CML patients undergoing treatment with imatinib can be ascribed to two main groups of mutations, namely, those that directly affect binding of imatinib to Abl and those that have an indirect binding effect. The latter mutations are thought to change the dynamics of the kinase domain in such a manner that the inactive, DFG-out, form is destabilized, resulting in a loss of affinity of imatinib for Abl (Table 2 and Figure 5a).

BCR-Abl Mutations That Affect the Gatekeeper and Hydrophobic Region. Mutations that directly affect the affinity of imatinib for Abl are the T315I/D/N, F317L, and G321W mutations. Mutations of Tyr 253 and Phe 359 probably have both a direct and an indirect effect on imatinib binding to Abl.

The residue Thr 315 in Abl is the so-called gatekeeper residue. The size of the residue at this position in kinases determines how easily the hydrophobic pocket behind it can be accessed, especially in the active form (DFG-in). In the case of imatinib binding, the hydroxyl group of Thr 315 forms a hydrogen bond to the amine linker between the pyrimidine and phenyl rings of imatinib. Mutation of the threonine thus results in a loss of this hydrogen bonding interaction, while the larger sizes of the residues at this position (isoleucine, aspartic acid, and asparagine) also result in steric clashes, preventing imatinib from binding to mutant Abl strutures (Figure 5a). Recent mutagenesis studies showed that gatekeeper mutations in Abl, PDGFRA,

Table 2. Partial List of Observed Resistance Mutations, Their Location, and the Mechanism of Resistance in Bcr-Abl, cKIT, PDGFR, and EGFR

	mechanism	Bcr-Abl	cKIT	PDGFR	EGFR
P-loop	activating	L248V/R, G250E, Q252H/R, Y253H/F, E255K/V			G719S
gatekeeper	direct except for EGFR	T315I/F/D/N	T670I/E	T674I	T790M
binding site	direct	F317L, G321W	V654A		
hydrophobic pocket	direct	M351T, F359V			
activation loop	activating	L387M, H396P/R	D816E, D820Y/E, N822Y/K, Y823D	D842V, D846Y	L858R
deletion mutations around helix C	activating				E746, L747, R748, E749, A750, T751, S752

PDGFRB, EGFR, and Src have increased activity and can transform BaF3 cells to become independent of IL-3 signaling for survival.[53] The increased activity is attributed to a strengthening of the "hydrophobic spine", which is a conserved feature of active kinases and is disrupted in inactive kinases.[54] The increase in activity can clearly further contribute to drug resistance.

The side chain of Phe 317, which is part of the hinge region, effectively forms the roof of the ATP binding site near the hinge region in the area where the critical hydrogen bonding interactions to the hinge region backbone are formed. The side chain shields the hinge region from solvent. This can be illustrated using the hydrophobic enclosure term of the Glide XP scoring function as shown in Figure 5b.[55] The role of this residue in forming a hydrophobic environment around the hinge region is further supported by an analysis of residue conservation at this position in ~480 kinases (alignments available at kinase.com). In ~300 kinases, an aromatic residue (Tyr, Phe, His, Trp) can be found at this position (62%) while small hydrophobic residues such as Ala, Ile, and Val are only found ~15 times (3%). Leucine is found in 25% of the kinases at this position, indicating that its packing properties are similar to those of tyrosine and phenylalanine in the context of the hinge region.

Similarly, Phe 359 forms the back of the DFG-out binding pocket and mutating it to a smaller, hydrophobic residue could also influence binding although the distance between Phe 359 and the inhibitor is beyond the van der Waals interaction sphere (~4.5 Å). It is possible that a mutation at this position also influences the dynamics of the region.

The Cα atom of Gly 321 in wild type (WT) Abl is 3.8 Å removed from the pyridine ring of imatinib (Figure 5a). Mutation to a larger residue such as tryptophan (G321W) would thus result in a direct clash with imatinib, resulting in loss of binding of imatinib to this mutant.

Tyrosine 253 in WT Abl presents a polar hydroxyl group to a region with a backbone-NH and an asparagine residue (distances 3.6 and 3.2 Å, respectively). The aromatic ring is 3.5−4.5 Å removed from the pyridine−pyrimidine rings of imatinib, possibly forming $\pi-\pi$ interactions. Both of these features likely play a role in binding, as mutations to phenylalanine result in loss of binding, as does mutation to histidine. In addition, this residue is part of the glycine-rich loop and the changes in the dynamics of the glycine-rich loop may play a significant role in the dynamics of the protein and the accessibility of the DFG-out vs DFG-in conformation of the catalytic domain.

Additional P-loop mutations have been observed at Gly 250, Gln 252, and Tyr 253. These are likely to affect the dynamics of the loop considerably, resulting in a different equilibrium between the active and inactive forms of the protein.

Leucine 248 in resistant tumors has been mutated to both a smaller residue (valine) and a larger one (arginine). The smaller residue may result in loss of binding due to a loss of van der Waals interactions with imatinib, while the larger residue will result in a significant clash with the pyridine ring (Figure 5a).

The E255K/V mutation probably influences the dynamics of the kinase catalytic domain. This residue is located directly after the glycine-rich loop. The glutamic acid side chain is placed within hydrogen-bonding distance of a hydroxyl group (from Tyr 257) and a charged nitrogen atom (from Lys 247). Mutating this residue to a lysine or valine will surely disrupt this network of hydrogen bonds (Figure 5a).

In summary, a large number of clinical mutations have been observed in response to imatinib treatment in CML. Some of these mutations appear to directly affect the binding of imatinib to Abl. However, most of these mutations decrease the occupation of the DFG-out form of the protein, which is the conformation of the protein that imatinib preferentially binds to. The shift of equilibrium away from this state is believed to be the key determinant of the loss in binding.

BCR-Abl Recent Clinical Candidates Demonstrating Drug Design Concepts To Circumvent Resistance. Since the development of imatinib, several new drugs have been developed that target Abl. The main goal of developing these additional drugs was to obtain greater efficacy, especially against the observed clinical mutations that confer resistance. Various mutant kinases can still bind imatinib with only a moderate loss in affinity of 10- to 30-fold, namely, those with Q252H, Y253F, M351T, F359V, or H396P mutations. The E255K mutation more severely affects imatinib inhibition of Abl, with a change in IC_{50} of 50-fold. The most severe losses in drug affinity are observed with the gatekeeper mutations T315I (3000-fold loss) and T315N ($IC_{50} > 10\ \mu M$).[56] Not surprisingly, the mutations with the greatest losses in potency have also been observed most frequently in patients who developed drug resistance on imatinib treatment.

Dasatinib maintains potent inhibition against most clinical mutations that are resistant to imatinib inhibition. This is possibly a consequence of the fact that it inhibits the DFG-in, or active, form of the Abl kinase, and many of the mutations are thought to destabilize the inactive form in favor of the active form of the kinase. Unfortunately, dasatinib is also highly sensitive to mutations of the gatekeeper residue, resulting in 80-fold (T315N) and 1200-fold (T315I) losses in potency, respectively.[56] Upon examination of the structure of dasatinib in complex with Abl the loss in potency upon gatekeeper residue mutation is not very surprising, as the inhibitor forms a hydrogen-bonding interaction with the threonine hydroxyl group (Figures 2 and 3a).

Nilotinib is structurally related to imatinib but is 30 times more potent than imatinib against cell lines expressing wild type Abl and mutated Abl.[57,58] Nilotinib forms the same hydrogen bonding interaction with the gatekeeper residue as imatinib and consequently shares its susceptibility to the T315I mutation. Significantly, this is the only imatinib-resistant mutant that is not inhibited by nilotinib.[57,58]

Although the marketed Abl inhibitors target different structural forms of the enzyme, none of them are able to inhibit the T315I mutant form of the enzyme, and thus, drug resistance is still a problem for CML patients. Current Abl inhibitors all form a hydrogen-bonding interaction with the threonine side chain of the gatekeeper residue, and thus, an obvious strategy to overcome resistance would be to develop inhibitors that do not interact with the gatekeeper residue at all. Early proof-of-concept was provided by modifying **2 (**AP23464, Figure 6) and removing the substituent that interacts with the region near the gatekeeper residue.[46] Although AP23846 (**3**) was less potent at inhibiting WT Abl, it did retain activity against the T315I mutant while **2** lost potency by more than 100-fold.

Further evidence of this strategy is provided by **4** (SGX70393 or SGX393;[59] undisclosed structure), which was described to have inhibitory activity against the T315I and other imatinib-resistant mutations in vitro and in mouse xenograft models. In addition, combination of **4** with nilotinib or dastinib completely repressed the emergence of known resistant clones in vitro.[59] Some insight into the binding is offered by cocrystal structures of **5** (PPY-A, Figure 6) available with both WT and T315I Abl.[41] This compound has been identified as an analogue of **4**. These structures show that the compound is 3 Å or more removed from the gatekeeper residue in either WT or the T315 mutant (Figure 7). Similarly, VX-680 (or MK-0457, **6**), originally developed as an Aurora kinase inhibitor,[60] also does not interact with the gatekeeper residue of Abl and has been shown in vitro[61] and clinically[62] to maintain activity against the imatinib-resistant T315I mutant.

An alternative strategy to overcome drug resistance would be the development of non-ATP competitive Abl inhibitors. ON012380 (**7**, Figure 6) has been described to inhibit 16 imatinib-resistant mutants in an in vitro setting.[63] No loss in potency was observed for inhibition of any of the mutant enzymes compared to the WT enzyme. Further experiments showed that this compound is not-ATP competitive but rather substrate competitive.

c-Kit. Expression of c-Kit, a receptor tyrosine kinase, occurs in 90% of gastrointestinal stromal tumors (GISTs).[64] In most cases of GIST, c-Kit exhibits activating mutations.[65] Until recently, the only treatment option was surgery, as GISTs do not respond to chemotherapy or radiation therapy. Upon identification of c-Kit as a molecular target of imatinib, the effect of imatinib on GIST was tested and a positive response was shown.[66] Subsequent clinical trials[67,68] led to FDA approval of imatinib in unresectable and/or metastatic GISTs.

X-ray crystallographic studies have shown that imatinib inhibits c-Kit by interacting with the DFG-out, inactive, form of the kinase. This is analogous to the mode of inhibition observed for imatinib with Abl.[69] As observed with CML patients, patients undergoing imatinib treatment for GIST eventually become resistant because of secondary mutations in c-Kit.

Over 10 imatinib-resistant mutations of 6 residues in the kinase domain have been described so far (Table 2).[70−74] Two of the observed mutations correspond to similar resistance mutants in Abl (Figure 4). Only two of the mutations in c-Kit are in proximity to the inhibitor and can influence the binding affinity directly, namely, T670I/E and V654A. Threonine 670 (Thr 315 in Abl) is the gatekeeper residue and forms a hydrogen bond with imatinib, which is lost upon mutation. As in Abl, this gatekeeper mutation leads to a complete loss of inhibition by imatinib.[56,75] Another common mutation in c-Kit is V654A, which is conserved in Abl (V299) but has not been observed to be mutated in imatinib-resistant CML. The valine side chain is ∼4 Å from imatinib, and mutation to alanine possibly leads to a less snug fit of the inhibitor resulting in resistance.

Other mutations mainly occur in the activation loop (D816E/H, D820V/Y/E, N822Y/K, Y823D) and are likely to increase the propensity of the enzyme to reside in the active form. The activation loop mutation at position 816 has a counterpart in Abl (Leu 387 in Abl). Both D816E and D816H are activating mutations, which have been linked to being the molecular cause of cancers such as acute myelogenous leukemia (AML)[43] and systemic mast cell disease (SMCD).[76] Mutation of Tyr 823 is possibly activating as well, as this is the tyrosine that is phosphorylated upon activation.

Similar to observations in Abl, dasatinib is more potent in WT c-Kit and several of the imatinib-resistant mutations. However, 1000-fold in potency is lost in the gatekeeper T670I mutant.[56,75]

PDGFR. While PDGFR plays a role in the development and maintenance of cancer because of its role in blood vessel growth, the validity of PDGFR as a drug target itself is unclear, as it is not usually the main factor in tumor development. However, there are several cancers that are more closely linked to up-regulation of PDGFR, namely, chronic myelomonocytic leukemia (CMML), GIST (when c-Kit mutations have not been observed), AML, chronic myeloproliferative disorders (CMPD), and dermatofibrossarcoma protruberans (DFSP), a soft tissue sarcoma. In some of these cancers, imatinib treatment has been successful, as it was shown that imatinib inhibits PDGFR at low-nanomolar concentrations.[77,78]

Different PDFGR fusion proteins are the underlying cause of DFSP[79,80] and CMML.[81,82] Clinical application of imatinib in patients with these cancers has resulted in responses that validate PDFGR as a target in these diseases and other cancers that are PDGFR-driven.[83−85]

While imatinib is effective in certain PDGFR-driven cancers, several known oncogenic mutations are resistant to imatinib treatment. The observed PDGFR mutations D842V and D846Y are equivalent to the cKIT D816E/H and D820V/Y mutations. These mutations are in the activation loop of the catalytic domain and lead to constitutively active kinases.[44,45] This suggests that imatinib inhibits PDGFR by the same mechanism as it inhibits Abl, namely, by binding to the inactive form of the kinase. To this date, no crystal structures of PDGFR have appeared in the public domain.

Targeted therapies such as imatinib can also be used to help further the understanding of cancers for which the molecular basis is unknown. For example, patients with hypereosinophilic syndrome (HES) showed dramatic clinical responses to imatinib treatment.[86−92] Later it was shown that a PDGFRα fusion protein underlies HES and that the fusion protein is indeed inhibited by imatinib.[93] Patients who developed resistance were shown to carry a mutation at T674I, which is equivalent to the T315I mutation in Abl.[93]

EGFR. The EGFR receptors have been oncology targets since the early days of kinase drug discovery. The epidermal growth factor (EGF) controls a pathway that is linked to cell proliferation, migration, and differentiation. The EGFR family of receptors contains four known members: EGFR-1 (later referred to as EGFR; also known as erythroblastic leukemia viral oncogene homologue 1, Erb-B1, or human EGF receptor HER-1), HER-2 (Erb-B2, also neurogliobastoma neu), HER-3 (Erb-B3), and HER-4 (Erb-B4). Of these, HER-2 lacks a known endogenous ligand and HER-3 lacks kinase activity.[94] Aberrant activity, either by overexpression or constitutive activation, has

been linked to a number of cancers, including lung, breast (especially HER-2/neu), and prostate cancers. Over the past decade extensive research, recently aided by inhibitors and crystallographic information, has progressively built a model of how the EGF signaling cascade operates.[94] Two drugs (erlotinib and gefitinib) have been approved for clinical use, both of which are designed to interact with the active form of the kinase. In 2007, lapatinib, a highly selective inhibitor of EGFR and HER-2, which binds the C-helix-out inactive form, was approved for breast cancer. Another generation of inhibitors, currently undergoing clinical trials, includes neratinib (**8**), pelitinib (**9**), canertinib (**10**), and BIBW-2992 (**11**) (Figure 8).

Clinical Mutations Affecting Inhibitor Binding. During clinical trials of getitinib and erlotinib for NSCLC, it was apparent that only a subset of the patient population with tumors showed a significant response to these drugs.[7,8,17−19,21,22,38,95] In one study with gefitinib, tumors from 119 patients with NSCLC were sequenced.[21] Two point mutations in the kinase domain, G719S and L858R, located in the P-loop and activation loop, respectively, were observed (Table 2). The somatic origin of these mutations was confirmed by sequencing normal lung tissue from the same patients. In addition, a number of deletion mutations in the region of residues 746−759 of the kinase domain were found. Analysis of the clinical data suggested that these mutations are activating, and patients harboring these mutations were more likely to respond to treatment with gefitinib. Similar conclusions were obtained in an analogous study.[20] Significantly, the enzymes with the L858R and G719S substitution mutations are both catalytically competent and are actually more active than the wild-type enzyme by 50- and 20-fold, respectively.[42]

The activating mutations, G719S and L858R, occur in the kinase domain regions that are critical to the binding of inhibitors. Kinetic parameters for ATP and a peptide substrate (poly-Glu_4Tyr_1) were measured in several assays for these mutants and the wild type enzyme. A comparison of the catalytic rate constants k_{cat} indicated that the L858R mutant is approximately 50-fold more active than the WT enzyme and that the G719S mutant is about 10-fold more active than WT. This greater activity of both the L858R and G719S mutants suggests a role of these activating mutations in shifting the conformation of the enzyme toward the active form.[42] Despite the enhancement of enzymatic activity of the L858R mutant, its apparent affinity for ATP as measured by the Michaelis−Menten constant K_m is reduced about 28-fold in comparison to the WT enzyme. This finding suggests that the L858R mutant is more easily inhibited by ATP-competitive inhibitors.[33] Thus, erlotinib and gefitinib succeed because the affinity for ATP is compromised by these mutations.

While substantial efforts have focused on understanding the relationship between ATP affinity and activity of mutant enzymes, kinetic data on mutant enzymes with peptide substrates have been limited to the L858R and G719S mutants. It is not surprising that the K_m value (443 μM) for the peptide substrate in the L858R mutant is about half that of the WT enzyme, since this residue lies in proximity to the peptide substrate binding site.[42] In contrast the substrate K_m value for the G719S mutant is similar to that of the WT enzyme.

In addition to the activating mutations discovered during clinical trials, another substitution mutation was uncovered by analysis of biopsies taken from NSCLC patients who initially responded to treatment with gefitinib or erlotinib but who later developed drug resistance.[7,96] Sequencing of the EGFR gene showed drug resistance was the result of the additional mutation of the gatekeeper threonine at position 790 to methionine (T790M). This mutation has been observed to occur concurrently with either the activating deletion mutants[7] or the substitution L858R mutation.[97]

The role of the T790M secondary mutation in drug resistance was elucidated on the basis of enzyme kinetics measurements complementing structural findings with inhibitors.[33] In this case, the K_m value for the T790M mutant does not significantly affect ATP affinity but activates the kinase about 5-fold in comparison to the WT enzyme.[33] These findings regarding ATP affinity and catalytic competency of the T790M mutant are in contrast to those of the L858R mutant and do not suggest any preference for the active or inactive conformation of the kinase. Indeed, two reported cocrystal structures of **12** (AEE788, Figure 8)[42] and **8**[33] bound to the T790M mutant were found in the active and inactive forms, respectively, supporting this conclusion. The K_m values for ATP in the WT (5.2 μM) and in the drug-resistant L858R/T790M double mutant (8.4 μM) are somewhat similar. However, the K_m value in the double mutant is 17-fold lower than in the single L858R mutant (148 μM). These data suggest a key role for the T790M mutation in restoring the ATP affinity back to the WT enzyme. Of interest is that the L858R/T790M mutant is also 10-fold more active than the WT enzyme and ∼5-fold more active than the L858R mutant based on a comparison of the specificity constant k_{cat}/K_m values.[33]

The sensitivity of kinases to reversible TKIs is greatly influenced by the affinity of the inhibitors for the enzyme, since these inhibitors must compete with high concentrations of ATP for binding in the cell. The binding of gefitinib to the L858R mutant (K_d = 2.4 nM) is 15-fold stronger than its binding to WT enzyme (K_d = 35.3 nM). Gefitinib also retains good binding affinity to the L858R/T790M double mutant (K_d = 10.9 nM). These data suggest that the clinically observed resistance of the L858R/T790M mutant is largely due to its enhanced affinity for ATP.[33]

EGFR Crystallography and Modeling. Considerable insight into the structural effect of mutations on the binding of inhibitors and ATP-like molecules has been provided by research undertaken by the Kuriyan[34,35] and Eck[7,33,42] laboratories, which has recently been reviewed. [98]

Crystal structures of EGFR in complex with the marketed drugs erlotinib[99] and gefitinib[42] both show the kinase in its active form. Figure 9 shows the location of bound gefitinib, as well as the relative disposition of the residues that confers resistance or oncogenic mutations. The inhibitor binds in the ATP cleft and accepts a key hydrogen bond from the backbone NH of Met 793. The 3-chloro-4-fluorophenyl group extends to a region adjacent to the catalytic lysine, making what appears to be van der Waals contacts to adjacent residues. With minor variations, other noncovalent inhibitors (such as erlotinib and **12**) share the same binding mode, location, and interactions, including the hydrogen bond to the bridging water.

Lapatinib binds to the C-helix-out inactive form of the kinase (Figure 10).[32] In this inactive form, Leu 858 is oriented into the binding cleft and forms part of the binding site. The C-helix shifts outward, opening additional space that is filled by the extended benzyloxy group on the inhibitor. The P-loop also moves closer to the binding site and forms additional contacts to the inhibitor. A crystal structure of this inactive form that binds ATP conjugated with a peptide in the substrate pocket has also been solved,[34] and this structure is essentially identical to the complex with lapatinib.

Gly 719 lies in the P-loop that forms part of the ATP binding cleft. Crystal structures have been obtained with the G719S

mutant bound to gefitinib and other inhibitors.[42] For gefitinib, this mutation does not appear to affect the binding mode. The mutation occurs in the region of, but not in contact with, the solubilizing morpholine group of the inhibitor. The effect of the G719S mutation is more interpretable when viewed in the context of the inactive form of the kinase. In this form, the conformation of the backbone of Gly 719 is one that is unique to glycine and inaccessible to other amino acids; mutation to serine or any other residue would lead to significant strain of this conformation and bias the P-loop to adopt another conformation (e.g., that of the active form).[98]

Gefitinib and other inhibitors have also been cocrystallized with the L858R mutant protein.[42] The observed binding modes are essentially unchanged compared to those obtained with the wild type enzyme. This is understandable because Leu 858 is located well away from the ATP binding site when in the active conformation.

The crystal structures of the inactive form of the kinase provide considerable insight into the mechanism of the activating mutation L858R. In this form of the kinase, Leu 858 is closely packed against several hydrophobic residues, including Leu 862, Met 766, Leu 777, Leu 788, and Leu 747. The L858R mutation would cause significant disruption of the inactive state due to steric clashes leading to changes in the shape of the binding site and by a severe mismatch of chemical properties.

Although crystallographic data for complexes of the deletion mutants are not available, some sense of their disposition with respect to the ATP site can be surmised. Residues preceding the C-helix that are the site of the oncogenic deletion mutations do not appear to be in contact with the inhibitors. The region from Glu 746 to Ser 752 is in a loop that traces an arc from the P-loop to the start of the C-helix. Truncation of three to five residues is likely to remove a turn from the C-helix while generally maintaining contacts as they are in the wild type enzyme.

Examination of the inactive form of the kinases provides additional insight into how the deletion mutants of the loop preceding the C-helix might disrupt the inactive state. Because the C-helix is shifted out compared to the orientation in the active form, this loop is drawn taut. The C-helix also contains one turn less than in the active form of the kinase. Truncation of this loop would lead to an even more strained system, probably leading to the unwinding of an additional turn of the C-helix. This could lead to a loss of interactions between secondary structural subunits, which understandably could destabilize the C-helix-out, inactive conformation.

Only one cocrystal structure of the T790M mutant in the active form is available, but it is remarkably informative.[33] When the T790M mutation was first identified as a resistance mutation, a number of authors attributed the resistance to a loss of binding affinity of the inhibitors due to direct steric interference and/or the possible loss of a bridging water interaction.[7,100] However, the structure of the **12** complex demonstrates that the mutated methionine can move to accommodate an inhibitor bound in the active form of the kinase.[42] Comparisons between this structure and the bound forms of gefitinib and erlotinib suggest that these compounds could also be accommodated in the T790M binding site. This is consistent with the observation that irreversible inhibitors, which otherwise are structurally similar to erlotinib and gefitinib, are able to inhibit the T790M mutant in conjunction with activating mutants.[97,101,102]

Irreversible Inhibitors. Relatively early on in researching inhibitors of EGFR kinase, two groups, at Wyeth and Parke-Davis, working independently pursued irreversible binding inhibitors of the enzyme.[101,103,104] A number of factors motivated these researchers to investigate inhibitors of this type. Among these was the expectation that such compounds might show a longer duration of action. Additionally, since it was expected that the major component of the inhibitory activity would be due to the covalent interaction, only those kinases that formed the covalent bond with the inhibitors should be significantly inhibited. This could result in an improvement in selectivity. A further motivation for designing these irreversible inhibitors was the thought that it would be difficult for a conventional reversibly binding inhibitor to compete with the high endogenous concentration of ATP within the cell for the extended time needed to exert effective antitumor activity. An irreversible inhibitor would be, in effect, noncompetitive with ATP and should thereby overcome this problem. While it turned out that reversible-binding inhibitors of EGFR could become useful drugs, the ability of these compounds to compete with ATP is a very important factor when considering the activity of inhibitors toward the mutant forms of the enzyme.

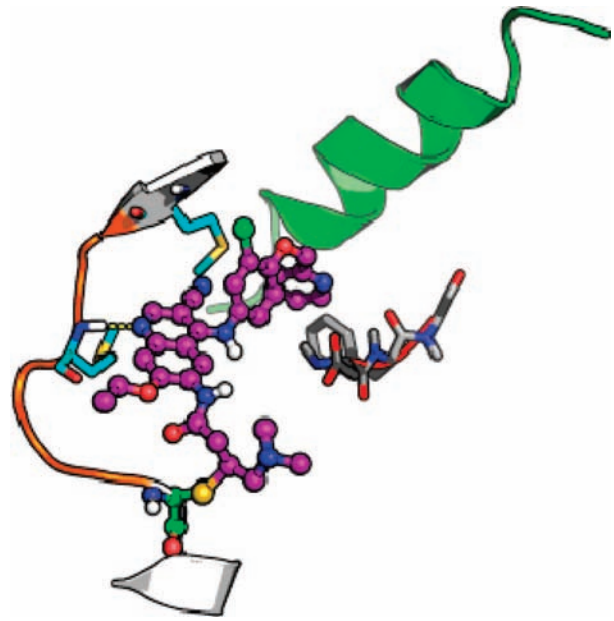

Figure 11. **8** bound to the T790M mutant of EGFR (2JIV.pdb). Cysteine 797 (green carbons) forms a covalent bond to the inhibitor (purple carbons).

A number of these irreversible inhibitors have been or are still in clinical trials targeting EGFR and/or HER-2 dependent cancers. Some of these compounds are shown in Figure 8. These compounds fall into two related series, either 4-anilinoquinazolines or 4-anilino-3-cyanoquinolines. The latter series was designed on the basis of the former to replace a predicted (and later observed) water-mediated hydrogen bond between the 3-N atom of a quinazoline inhibitor and the protein by a direct hydrogen bond to the protein with the 3-cyano group.[105] These compounds function as irreversible inhibitors by forming a covalent interaction with a cysteine residue conserved within the ErbB kinase family. The covalent bond is the result of a Michael addition reaction between the inhibitor and the sulfhydryl group of Cys 797 in EGFR(or Cys 773, using the alternative EGFR numbering scheme that excludes a signal sequence) or Cys 805 in HER-2. The crystal structure of **8** was solved in complex with the T790M mutant and clearly showed the covalent bond between the reactive cysteine and the Michael acceptor (Figure 11).

A particular advantage of these irreversible inhibitors over the first generation of EGFR inhibitors such as erlotinib and

gefitinib is their ability to retain inhibitory efficacy against cancers that have mutated and become resistant to the first generation of drugs. This has become evident from both studies in cell culture and from some interim results of ongoing clinical trials. For example, in cells with the activating L747-S752 deletion mutation and those with the L747-S752 deletion plus T790M mutation, the irreversible inhibitor EKI-785 (**13**) showed significant inhibitory activity against both cells lines while erlotinib was ineffective against the cell line having the double mutation.[7] Similarly, in cell lines having the L858R/T790M double mutation, **8**−**11** showed potent inhibitory activity, whereas gefitinib and erlotinib were ineffective.[5,97,106] Most significantly, irreversible inhibitors such as **8** have shown efficacy during clinical trials in treating NSCLC patients with disease that has become resistant to conventional ATP-competitive inhibitors.[102]

The activity of gefitinib, erlotinib, and **8** was evaluated in Ba/F3 cells transformed with the L858R and L858R/T790M mutations. In cells transformed with L858R mutants, gefitinib, erlotinib, and **8** maintain their sensitivity (IC_{50} values of 10.8, 12.5, and 3.5 nM, respectively); however, cells transformed with the double mutant are not inhibited by gefitinib or erlotinib but retain sensitivity to **8** (IC_{50} = 180 nM).[18] Moreover, in a mouse lung model that is dependent on activated EGFR signaling, erlotinib and **8**, as well as the humanized anti-hEGFR antibody cetuximab, led to dramatic tumor regression.[38] It is noted that while the irreversible inhibitors such as **8** can overcome primary resistance mutations by forming a covalent interaction with an active site cysteine, such drugs could conceivably induce a secondary resistance by mutation of this cysteine. At this time, clinical experience with these inhibitors is limited and it remains to be seen if this additional resistance mechanism will emerge.

An X-ray crystal structure of the clinical candidate **8** in complex with the T790M single mutant kinase has recently been disclosed.[33] **8** interacts with the C-helix-out inactive form in a binding mode that is similar to that of lapatinib except for the addition of a covalent bond with Cys 797. Consistent with the observations arising from the kinetics of mutant receptors, the side chain of mutant Met 790 adopted a conformation that did not perturb the binding of **8**. In doing so, it makes hydrophobic contacts with Cys 775 and Leu 777. Presumably, these additional hydrophobic contacts may lead to some restoration of wild-type affinity for ATP when introduced into a kinase with an activating mutant.[33,98] Much as with lapatinib complex, the additional 2-pyridylmethoxy group extends into the space created by the outward movement of the C-helix. The incompatibility of the active state with **8** binding coupled with **8**'s persistent ability to inhibit kinases with the L858R/T790M double mutation implies that a conformation of the kinase similar to the C-helix-out form is available in the double mutant. However, this remains speculative because it is not clear by how much the L858R or the deletion mutants (e.g., del 746−750) disrupt the inactive state.

3. Drug Design Strategies

Our current knowledge about kinase domain mutations and the need to overcome drug resistance due to these mutations is a compelling driver for drug discovery within oncology. The preceding section has identified several strategies that have led to the next generation of kinase inhibitors. Some of these inhibitors have been shown to have better efficacy against known mutants and/or better risk profiles against the emergence of new mutant strains. In this section, we will review the key strategies needed to develop drugs that overcome drug resistance, highlighting both what has already been achieved and the remaining issues.

Clearly, the simplest and most important strategy to overcome resistance is to increase the potency of the inhibitors toward their target proteins. Many of the resistance mutations reduce drug-binding affinity and therefore drug inhibitory activity sufficiently to allow continued cancer growth. Although in absolute terms this represents a challenge for a given drug, in relative terms, the effect on ligand binding for most mutations is not as severe as might be anticipated, often only 10- to 30-fold. Therefore, a more potent analogue of a clinical drug may overcome many clinical resistant mutations. This is illustrated by nilotinib, which was designed as a successor to imatinib and demonstrates 10-fold more potent binding to the wild type enzyme. This is responsible for its effectiveness against all but the T315I mutation in BCR-Abl. For any given chemical class, the ability to increase in vivo potency involves a balance of factors, namely, changing the molecule to increase or improve molecular interactions with the kinase while maintaining or improving physicochemical and pharmacokinetic properties. While reaching this strategic objective may not be successful in a parent series of inhibitors, identifying a new chemical series or making significant structural changes to an existing series (with its attendant risks and uncertainties) may be necessary.

As the development of **8**−**11** have demonstrated, irreversible inhibitors that form a covalent interaction with an active site cysteine residue are viable drug candidates. Effectively, this can be considered as a special case of increasing the potency of the drug. Yun et al. estimated that the potency of a covalent inhibitor is equivalent to a 0.2 nM inhibitor.[107] Additionally, irreversible inhibition requires receptor cycling to regenerate active enzyme. This could result in sustained inhibitory activity for this class of compounds. Importantly, irreversible inhibitors do not compete with high ATP concentrations in the cell and are additionally unaffected by changes in the ATP binding affinity that can occur with mutant forms of the enzyme. It is hoped that concerns about toxicity caused by nonspecific reactivity will be mitigated provided that the reactive moiety can be tuned to have an appropriate degree of reactivity and orientation with respect to the target residue. Assuming that the clinical experience to date continues through FDA approval resulting in clinical utility, the key limit to this strategy is opportunity. While there are only 10 kinases including EGFR with a homologous cysteine, other kinases have cysteines at other locations in the binding site. These appear to function as small hydrophobic residues and can serve as targets for irreversible inhibitors. A recent analysis suggested that there were over 200 kinases with cysteines accessible to drug targeting.[108] Still, the irreducible minimum for this strategy to succeed is the presence of a binding site cysteine.

Another strategy to overcome resistance is to rationally design inhibitors to avoid or co-opt specific mutations. Clearly, in Bcr-Abl, the gatekeeper threonine makes a direct and important hydrogen bond to imatinib and nilotinib. Upon mutation, this bond is lost, essentially leading to a complete loss of drug potency. The design of inhibitors that avoid a direct interaction with the gatekeeper or avoid sterically clashing with the gatekeeper upon mutation has been shown to be a viable strategy. Sunitinib has recently been shown to be, surprisingly, a type II inhibitor, with its continued effectiveness in cells with the gatekeeper mutation T670I due to a lack of interaction with Thr 670.[109] In preclinical studies, compounds with this property such as **6** have been identified by directed screening of mutant

kinases.[5] Similarly, **4** was the result of a discovery effort with this strategy in mind from the beginning.

The realization that the resistance effects of the gatekeeper mutation in EGFR are not solely due to direct interactions with the ligand but are the result of enhancing the binding affinity for ATP argues against adopting this uncritically as a universal strategy. Indeed, it is this property of the L858R/T790M double mutation of EGFR kinase that is primarily responsible for the resistance. In contrast, in Abl the gatekeeper mutation leads to a direct loss of inhibitor potency due to the loss of a hydrogen bonding interaction. The fact that seemingly similar mutations in Abl and EGFR result in different mechanisms of drug resistance highlights the need to fully characterize the mutant enzymes both kinetically and structurally to be able to identify an appropriate drug design strategy to overcome resistance.

Where there is clear clinical evidence of mutations, there is also the opportunity to rationally design inhibitors of the mutant enzymes. This could involve exploiting a significant change in the shape or character of the binding site. The means by which a ligand could be designed in this way are numerous: (a) a focused screening effort could be developed to identify templates that preferentially bind to the mutant rather than the native enzyme;[31] (b) a known template could be rationally modified with substituents that exploit the novel shape or character of the binding pocket; or (c) fragment screening might be employed to identify moieties that uniquely bind to the novel pocket. An emerging opportunity to support this strategy is the identification of potential mutations in vitro early in the drug design process.[110−113]

Another strategy is to rationally design inhibitors for the different structural states of a kinase. As we have highlighted, a number of the known resistance mutations bias the structures of the Abl and c-Kit enzymes from the inactive to the active state, thereby reducing the binding affinity of those inhibitors, such as imatinib, that prefer the inactive form of the enzyme. Dasatinib, which binds to the active state of the kinase, appears to be less sensitive to such mutations for that reason. Similarly, gefitinib and erlotinib appear to be uniquely effective against mutant EGFR kinases that favor the active state.

A clinical treatment strategy to prevent or slow the occurrence of drug resistance could be to administer two drugs having different mechanisms of action simultaneously. At least in an in vitro setting this seems like a promising avenue.[59]

Crystallographic knowledge derived from multiple crystal structures in various conformational states of EGFR, Abl, cKIT, and other kinases is a valuable asset for optimal inhibitor design. The value of such an asset is amplified when kinetic analyses of mutant enzymes specifically in relation to ATP affinity and catalytic competence are revealed. Such knowledge complements that obtained by crystallography at the molecular level and should pave the road for designing inhibitors with desired profiles.

4. Conclusion

The identification of kinase domain mutations in the clinic has stimulated basic research efforts to relate structure and function as reviewed in this Perspective. The specific properties of these mutations can influence the structural states, binding site character, and affinity for ATP of the kinases. Understanding these aspects in combination will be pivotal for any drug design program with the goal of identification of a small molecule inhibitor that can overcome resistance mechanisms. Protein structural information obtained from multiple crystal structures in various conformational states is a valuable asset for optimal inhibitor design. This information can be further enhanced by kinetic analyses of mutant enzymes with respect to ATP affinity and catalytic competence. The clinical need and preclinical complexity of the design challenge both point to adopting the most integrated approach available when designing the next generation of inhibitors.

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Biographies

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References

(1) Manning, G.; Whyte, D. B.; Martinez, R.; Hunter, T.; Sudarsanam, S. The protein kinase complement of the human genome. *Science*

2002, *298* (5600), 1912–1934.
(2) LaRusso, P. M.; Eder, J. P. Therapeutic potential of novel selective-spectrum kinase inhibitors in oncology. *Expert Opin. Invest. Drugs* **2008**, *17* (7), 1013–1028.
(3) Liao, J. J.-L. Molecular recognition of protein kinase binding pockets for design of potent and selective kinase inhibitors. *J. Med. Chem.* **2007**, *50* (3), 409–424.
(4) Liao, J. J.-L.; Andrews, R. C. Targeting protein multiple conformations: a structure-based strategy for kinase drug design. *Curr. Top. Med. Chem.* **2007**, *7*, 1394–1407.
(5) Carter, T. A.; Dodicka, L. M.; Shah, N. P.; Velasco, A. M.; Fabian, M. A.; Treiber, D. K.; Milanov, Z. V.; Atteridge, C. E.; Biggs, W. H.; Edeen, P. T.; Floyd, M.; Ford, J.; Grotzfeld, R. M.; Herrfard, S.; Insko, D. E.; Mehta, S. A.; Paetel, H. K.; Pao, W.; Sawyers, C. L.; Varmus, H.; Zarinkar, P. P.; Lockhart, D. J. Inhibition of drug-resistant mutants of ABL, KIT, and EGF receptor kinases. *Proc. Natl. Acad. Sci. U.S.A.* **2005**, *102* (31), 11011–11016.
(6) Daub, H.; Specht, K.; Ullrich, A. Strategies to overcome resistance to targeted protein kinase inhibitors. *Nat. Rev. Drug Discovery* **2004**, *3* (12), 1001–1010.
(7) Kobayashi, S.; Boggon, T. J.; Dayaram, T.; Janne, P.; Kocher, O.; Meyerson, M.; Johnson, B. E.; Eck, M. J.; Tenen, D. G.; Halmos, B. EGFR mutation and resitance of non-small-cell lung cancer to gefitinib. *N. Engl. J. Med.* **2005**, *352* (8), 786–792.
(8) Dowell, J. E.; Minna, J. D. Chasing mutations in the epidermal growth factor in lung cancer. *N. Engl. J. Med.* **2005**, *352* (8), 830–832.
(9) Gorre, M. E.; Mohammed, M.; Ellwood, K.; Hsu, N.; Paquette, R. L.; Rao, P. N.; Sawyers, C. L. Clinical resistance to STI-571 cancer therapy caused by BCR-ABL gene mutation or amplification. *Science* **2001**, *293*, 876–880.
(10) Engelman, J. A.; Zejnullahu, K.; Misudomi, T.; Song, Y.; Hyland, C.; Park, J. O.; Lindeman, N.; Gale, C.-M.; Zhao, X.; Christensen, J.; Kosaka, T.; Holmes, A., J; Rogers, A. M.; Cappuzzo, F.; Mok, T.; Lee, C.; Johnson, B. E.; Cantley, L. C.; Janne, P. A. MET amplification leads to gefitinib resistance in lung cancer by activating ERBB3 signaling. *Science* **2007**, *316* (5827), 1039–1043.
(11) Quintas-Cardama, A.; Cortes, J. Therapeutic options against BCR-ABL1 T315I-positive chronic myelogenous leukemia. *Clin. Cancer Res.* **2008**, *14* (14), 4392–4399.
(12) Druker, B. J.; Tamaru, S.; Buchdunger, E.; Ohno, S.; Segal, G. M.; Zimmermann, J.; Lydon, N. B. Effects of a selective inhibitor of the Abl tyrosine kinase on the growth of Bcr-Abl positive cells. *Nat. Med.* **1996**, *2*, 561–566.
(13) Boschelli, D. H. Bcr-Abl kinase inhibitors. *Top. Med. Chem.* **2007**, *1*, 407–444.
(14) Noronha, G.; Cao, J.; Chow, C. P.; Dneprovskaia, E.; Fine, R. M.; Hood, J.; Kang, X.; Klebansky, B.; Lohse, D.; Mak, C. C.; McPherson, A.; Moorthy, S. S. P.; Pathak, V. P.; Renick, J.; Soll, R.; Zeng, B. Inhibitors of ABL and the ABL-T315I mutation. *Curr. Top. Med. Chem.* **2008**, *8*, 905–921.
(15) Quintas-Cardama, A.; Kantarijian, H.; Cortes, J. Flying under the radar: the new wave of BCR-ABL inhibitors. *Nat. Rev. Drug Discovery* **2007**, *6* (10), 834–848.
(16) Chan, S. K.; Gullick, W. J.; Hill, M. E. Mutations of the epidermal growth factor receptor in non-small cell lung cancer—search and destroy. *Eur. J. Cancer* **2006**, *42*, 17–23.
(17) Gazdar, A. F.; Shigematsu, H.; Herz, J.; Minna, J. D. Mutations and addiction to EGFR: the Achilles "heal" of lung cancers. *Trends Mol. Med.* **2004**, *10* (10), 481–486.
(18) Ji, H.; Zhao, X.; Yuza, Y.; Shimamura, T.; Li, D.; Protopopov, A.; Jung, B. L.; McNamara, K.; Xia, H.; Glatt, K. A.; Thomas, R. K.; Sasaki, H.; Horner, J. W.; Eck, M. J.; Discafani, C. M.; Maher, E.; Shapiro, G. I.; Meyerson, M.; Wong, K.-K. Epidermal growth factor receptor variant III mutations in lung tumorigenesis and sensitivity to tyrosine kinase inhibitors. *Proc. Natl. Acad. Sci. U.S.A.* **2006**, *103* (20), 7817–7822.
(19) Johnson, B. E.; Janne, P. Epidermal growth factor receptor mutations in patients with non-small cell lung cancer. *Cancer Res.* **2005**, *65* (17), 7525–7529.
(20) Lynch, T. J.; Bell, D. W.; Sordella, R.; Gurubhagavatual, S.; Okimoto, R. A.; Brannigan, B. W.; Harris, P. L.; Haserlat, S. M.; Supko, J. G.; Haluska, F. G.; Louis, D. N.; Christiani, D. C.; Settleman, J.; Haber, D. A. Activating mutations in the epidermal growth factor receptor underlying responsiveness of non-small-cell lung cancer to gefitinib. *N. Engl. J. Med.* **2004**, *350* (21), 2129–2139.
(21) Paez, J. G.; Janne, P. A.; Lee, J. C.; Tracy, S.; Greulich, H.; Gabriel, S.; Herman, P.; Kaye, F. J.; Lindeman, N.; Boggon, T. J.; Naoki, K.; Sasaki, H.; Fujii, Y.; Eck, M. J.; Sellers, W. R.; Johnson, B. E.; Meyerson, M. EGFR mutations in lung cancer: correlation with clinical response to gefitinib therapy. *Science* **2004**, *304*, 1497–1500.
(22) Pao, W.; Miller, V. A.; Politi, K. A.; Reily, G. I.; Somwar, R.; Zakowski, M. F. EGF receptor gene mutations are common in lung cancers from "never smokers" and are associated with sensitivity of tumors to gefitinib and erlotinib. *Proc. Natl. Acad. Sci. U.S.A.* **2004**, *101*, 13306–13311.
(23) Faivre, S.; Demetri, G.; Sargent, W.; Raymond, E. Molecular basis for sunitinib efficacy and future clinical development. *Nat. Rev. Drug Discovery* **2007**, *6* (9), 734–745.
(24) Sun, L.; Liang, C.; Shirazian, S.; Zhou, T.; Miller, T.; Cui, J.; Fukuda, J. Y.; Chu, J.-Y.; Nematalla, A.; Wang, X.; Chen, H.; Wei, J.; Tang, C. Discovery of 5-[5-fluoro-2-oxo-1,2-dihydroindol-(3*Z*)-ylidenemethyl]-2,4-dimethyl-1*H*-pyrrole-3-carboxylic acid (2-diethylaminoethyl)amide, a novel tyrosine kinase inhibitor targeting vascular endothelial and platelet-derived growth factor receptor tyrosine kinase. *J. Med. Chem.* **2003**, *46* (7), 1116–1119.
(25) Lowinger, T.; Riedl, B.; Dumas, J.; Smith, R. A. Design and discovery of small molecules targeting Raf-1 kinase. *Curr. Pharm. Des.* **2002**, *8* (25), 2269–2278.
(26) Huse, M.; Kuriyan, J. The conformational plasticity of protein kinases. *Cell* **2002**, *109* (3), 275–282.
(27) Liu, Y.; Gray, N. S. Rational design of inhibitors that bind to inactive kinase conformations. *Nat. Chem. Biol.* **2006**, *2* (7), 358–364.
(28) Masterson, L. R.; Mascioni, A.; Traaseth, N. J.; Taylor, S. S.; Veglia, G. Allosteric cooperativity in protein kinase A. *Proc. Natl. Acad. Sci. U.S.A.* **2008**, *105* (2), 506–511.
(29) Vajpai, N.; Strauss, A.; Fendrich, G.; Cowan-Jacob, S. W.; Manley, P. W.; Grzesiek, S.; Jahnke, W. Solution conformations and dynamics of ABL kinase-inhibitor complexes determined by NMR substantiate the different binding modes of imatinib/nilotinib and dasatinib. *J. Biol. Chem.* **2008**, *283* (26), 18292–18302.
(30) Dorsey, J. F.; Jove, R.; Kraker, A. J.; Wu, J. The pyrido[2,3-*d*]pyrimidine derivative PD180970 inhibits p210Bcr-Abl tyrosine kinase and induces apoptosis of K562 leukemic cells. *Cancer Res.* **2000**, *60* (12), 3127–3131.
(31) Kraker, A. J.; Hartl, B. G.; Amar, A. M.; Barvian, M. R.; Showalter, H. D. H.; Moore, C. W. Biochemical and cellular effects of c-Src kinase-selective pyrido[2,3-*d*]pyrimidine tyrosine kinase inhibitors. *Biochem. Pharmacol.* **2000**, *60* (7), 885–898.
(32) Wood, E. R.; Tuesdale, A. T.; McDonald, O. B.; Yan, D.; Hassell, A.; Dickerson, S. H.; Ellis, B.; Pennisi, C.; Horne, E.; Lakcey, K.; Alligood, K. J.; Rusnak, D. W.; Gilbmer, T. M.; Shewchuck, L. A unique structure for epidermal growth factor receptor bound to GW572016 (lapatinib): relationships among protein conformation, inhibitor off-rate, and receptor activity in tumor cells. *Cancer Res.* **2004**, *64*, 66452–66659.
(33) Yun, C.-H.; Mengwasser, K. E.; Toms, A. V.; Woo, M. S.; Greulich, H.; Wong, K.-K.; Meyerson, M.; Eck, M. J. The T790M mutation in EGFR kinase causes drug resistance by increasing the affinity for ATP. *Proc. Natl. Acad. Sci. U.S.A.* **2008**, *105*, 2070–2075.
(34) Zhang, X.; Gureasko, J.; Shen, H.; Cole, P. A.; Kuriyan, J. An allosteric mechanism for activation of the kinase domain of epidermal growth factor receptor. *Cell* **2006**, *125*, 1137–1149.
(35) Zhang, X.; Pickin, K. A.; Bose, R.; Jura, N.; Cole, P. A.; Kuriyan, J. Inhibition of the EGF receptor by binding of MIG6 to an activating kinase domain interface. *Nature* **2007**, *450*, 741–745.
(36) Levinson, N. M.; Kuchment, O.; Shen, K.; Young, M. A.; Koldobskiy, M.; Karplus, M.; Cole, P. A.; Kuriyan, J. A Src-like inactive conformation in the Abl tyrosine kinase domain. *PLoS Biol.* **2006**, *4* (5), 753–767.
(37) Seeliger, M. A.; Nagar, B.; Frank, F.; Coa, X.; Henderson, M. N.; Kuriyan, J. c-Src binds to the cancer drug imatinib with an inactive Abl/c-Kit conformation and a distributed thermodynamic penalty. *Structure* **2007**, *15*, 299–311.
(38) Ji, H.; Li, D.; Chen, L.; Shimamura, T.; Kobayahshi, S.; McNamara, K.; Mahmood, U.; Mitchell, A.; Sun, Y.; Al-Hashem, R.; Chirieac, L. R.; Padera, R.; Bronson, R. T.; Kim, W.; Janne, P.; Shapiro, G. I.; Tenen, D. G.; Johnson, B. E.; Weissleder, R.; Sharpless, N. E.; Wong, K.-K. The impact of human EGFR kinase domain mutations on lung tumorigenesis and in vivo sensitivity to EGFR-targeted therapies. *Cancer Cell* **2006**, *9*, 485–495.
(39) Modugno, M.; Casale, E.; Soncini, C.; Rosettani, P.; Colombo, R.; Lupi, R.; Rusconi, L.; Fancelli, D.; Carpinelli, P.; Cameron, A. D.; Isacchi, A.; Moll, J. Crystal structure of the T315I Abl mutant in complex with the Aurora kinase inhibitor PHA-739358. *Cancer Res.* **2007**, *67* (17), 7987–7990.
(40) Young, M. A.; Shah, N. P.; Chao, L. H.; Seeliger, M.; Milanov, Z. V.; Biggs, W. H., 3rd; Treiber, D. K.; Patel, H. K.; Zarrinkar, P. P.; Lockhart, D. J.; Sawyers, C. L.; Kuriyan, J. Structure of the kinase domain of an imatinib-resistant Abl mutant in complex with the Aurora kinase inhibitor VX-680. *Cancer Res.* **2006**, *66* (2), 1007–1014.
(41) Zhou, T.; Parillon, L.; Li, F.; Wang, Y.; Keats, J.; Lamore, S.; Xu, Q.; Shakespeare, W.; Dalgarno, D.; Zhu, X. Crystal structure of the T315I mutant of Abl kinase. *Chem. Biol. Drug Des.* **2007**, *70* (3), 171–181.

(42) Yun, C.-H.; Boggon, T. J.; Li, Y.; Woo, M. S.; Greulich, H.; Meyerson, M.; Eck, M. J. Structures of lung cancer-derived EGFR mutants and inhibitor complexes: mechanism of activation and insights into differential inhibitor sensitivity. *Cancer Cell* **2007**, *11*, 217–227.
(43) Beghini, A.; Cairoli, R.; Morra, E.; Larizza, L. In vivo differentiation of mast cells from acute myeloid leukemia blasts carrying a novel activating ligand-independent C-kit mutation. *Blood Cells, Mol. Dis.* **1998**, *24* (2), 262270.
(44) Chompret, A.; Kannengiesser, C.; Barrois, M.; Terrier, P.; Dahan, P.; Tursz, T.; Lenoir, G. M.; Bressac-De Paillerets, B. PDGFRA germline mutation in a family with multiple cases of gastrointestinal stromal tumor. *Gastroenterology* **2004**, *126* (1), 318–321.
(45) Heinrich, M. C.; Corless, C. L.; Duensing, A.; McGreevey, L.; Chen, C. J.; Joseph, N.; Singer, S.; Griffith, D. J.; Haley, A.; Town, A.; Demetri, G. D.; Fletcher, C. D.; Fletcher, J. A. PDGFRA activating mutations in gastrointestinal stromal tumors. *Science* **2003**, *299* (5607), 708–710.
(46) Azam, M.; Nardi, V.; Shakespeare, W. C.; Metcalf, C. A., III; Bohacek, R. S.; Wang, Y.; Sundaramoorthi, R.; Sliz, P.; Veach, D. R.; Bornmann, W. G.; Clarkson, B.; Dalgarno, D. C.; Sawyer, T. K.; Daley, G. Q. Activity of dual SRC-ABL inhibitors highlights the role of BCR/ABL kinase dynamics in drug resistance. *Proc. Natl. Acad. Sci. U.S.A.* **2006**, *103* (24), 9244–9249.
(47) Hochhaus, A.; Kreil, S.; Corbin, A. S.; La Rosee, P.; Muller, M. C.; Lahaye, T.; Hanfstein, B.; Schoch, C.; Cross, N. C.; Berger, U.; Gschaidmeier, H.; Druker, B. J.; Hehlmann, R. Molecular and chromosomal mechanisms of resistance to imatinib (STI571) therapy. *Leukemia* **2002**, *16* (11), 2190–2196.
(48) Roumiantsev, S.; Shah, N. P.; Gorre, M. E.; Nicoll, J.; Brasher, B. B.; Sawyers, C. L.; Van Etten, R. A. Clinical resistance to the kinase inhibitor STI-571 in chronic myeloid leukemia by mutation of Tyr-253 in the Abl kinase domain P-loop. *Proc. Natl. Acad. Sci. U.S.A.* **2002**, *99* (16), 10700–10705.
(49) Shah, N. P.; Nicoll, J. M.; Nagar, B.; Gorre, M. E.; Paquette, R. L.; Kuriyan, J.; Sawyers, C. L. Multiple BCR-ABL kinase domain mutations confer polyclonal resistance to the tyrosine kinase inhibitor imatinib (STI571) in chronic phase and blast crisis chronic myeloid leukemia. *Cancer Cell* **2002**, *2* (2), 117–125. [Comment].
(50) O'Hare, T.; Eide, C. A.; Deininger, M. W. Bcr-Abl kinase domain mutations and the unsettled problem of Bcr-AblT315I: looking into the future of controlling drug resistance in chronic myeloid leukemia. *Clin. Lymphoma Myeloma* **2007**, *7* (Suppl. 3), S120–S130.
(51) Soverini, S.; Colarossi, S.; Gnani, A.; Rosti, G.; Castagnetti, F.; Poerio, A.; Iacobucci, I.; Amabile, M.; Abruzzese, E.; Orlandi, E.; Radaelli, F.; Ciccone, F.; Tiribelli, M.; di Lorenzo, R.; Caracciolo, C.; Izzo, B.; Pane, F.; Saglio, G.; Baccarani, M.; Martinelli, G.; Leukemia, G. W. P. o. C. M. Contribution of ABL kinase domain mutations to imatinib resistance in different subsets of Philadelphia-positive patients: by the GIMEMA Working Party on Chronic Myeloid Leukemia. *Clin. Cancer Res.* **2006**, *12* (24), 7374–7379.
(52) Nagar, B.; Bornmann, W. G.; Pellicena, P.; Schindler, T.; Veach, D. R.; Miller, W. T.; Clarkson, B.; Kuriyan, J. Crystal structures of the kinase domain of c-Abl in complex with the small molecule inhibitors PD173955 and imatinib (STI-571). *Cancer Res.* **2002**, *62* (15), 4236–4243.
(53) Azam, M.; Seeliger, M. A.; Gray, N. S.; Kuriyan, J.; Daley, G. Q. Activation of tyrosine kinases by mutation of the gatekeeper threonine. *Nat. Struct. Mol. Biol.* **2008**, *15* (10), 1109–1118.
(54) Kornev, A. P.; Haste, N. M.; Taylor, S. S.; Eyck, L. F. Surface comparison of active and inactive protein kinases identifies a conserved activation mechanism. *Proc. Natl. Acad. Sci. U.S.A.* **2006**, *103*, 17783–17788.
(55) Friesner, R. A.; Murphy, R. B.; Repasky, M. P.; Frye, L. L.; Greenwood, J. R.; Halgren, T. A.; Sanschagrin, P. C.; Mainz, D. T. Extra precision glide: docking and scoring incorporating a model of hydrophobic enclosure for protein−ligand complexes. *J. Med. Chem.* **2006**, *49* (21), 6177–6196.
(56) Carter, T. A.; Wodicka, L. M.; Shah, N. P.; Velasco, A. M.; Fabian, M. A.; Treiber, D. K.; Milanov, Z. V.; Atteridge, C. E.; Biggs, W. H.; Edeen, P. T.; Floyd, M.; Ford, J. M.; Grotzfeld, R. M.; Herrgard, S.; Insko, D. E.; Mehta, S. A.; Patel, H. K.; Pao, W.; Sawyers, C. L.; Varmus, H.; Zarrinkar, P. P.; Lockhart, D. J. Inhibition of drug-resistant mutants of ABL, KIT, and EGF receptor kinases. *Proc. Natl. Acad. Sci. U.S.A.* **2005**, *102* (31), 11011–11016.
(57) O'Hare, T.; Walters, D. K.; Stoffregen, E. P.; Jia, T.; Manley, P. W.; Mestan, J.; Cowan-Jacob, S. W.; Lee, F. Y.; Heinrich, M. C.; Deininger, M. W.; Druker, B. J. In vitro activity of Bcr-Abl inhibitors AMN107 and BMS-354825 against clinically relevant imatinib-resistant Abl kinase domain mutants. *Cancer Res.* **2005**, *65* (11), 4500–4505.
(58) le Coutre, P.; Ottmann, O. G.; Giles, F.; Kim, D.-W.; Cortes, J.; Gattermann, N.; Apperley, J. F.; Larson, R. A.; Abruzzese, E.; O'Brien, S. G.; Kuliczkowski, K.; Hochhaus, A.; Mahon, F.-X.; Saglio, G.; Gobbi, M.; Kwong, Y.-L.; Baccarani, M.; Hughes, T.; Martinelli, G.; Radich, J. P.; Zheng, M.; Shou, Y.; Kantarjian, H. Nilotinib (formerly AMN107), a highly selective BCR-ABL tyrosine kinase inhibitor, is active in patients with imatinib-resistant or -intolerant accelerated-phase chronic myelogenous leukemia. *Blood* **2008**, *111* (4), 1834–1839.
(59) O'Hare, T.; Eide, C. A.; Tyner, J. W.; Corbin, A. S.; Wong, M. J.; Buchanan, S.; Holme, K.; Jessen, K. A.; Tang, C.; Lewis, H. A.; Romero, R. D.; Burley, S. K.; Deininger, M. W. SGX393 inhibits the CML mutant Bcr-AblT315I and preempts in vitro resistance when combined with nilotinib or dasatinib. *Proc. Natl. Acad. Sci. U.S.A.* **2008**, *105* (14), 5507–5512.
(60) Harrington, E. A.; Bebbington, D.; Moore, J.; Rasmussen, R. K.; Ajose-Adeogun, A. O.; Nakayama, T.; Graham, J. A.; Demur, C.; Hercend, T.; Diu-Hercend, A.; Su, M.; Golec, J. M. C.; Miller, K. M. VX-680, a potent and selective small-molecule inhibitor of the Aurora kinases, suppresses tumor growth in vivo. *Nat. Med.* **2004**, *10* (3), 262–267.
(61) Shah, N. P.; Skaggs, B. J.; Branford, S.; Hughes, T. P.; Nicoll, J. M.; Paquette, R. L.; Sawyers, C. L. Sequential ABL kinase inhibitor therapy selects for compound drug-resistant BCR-ABL mutations with altered oncogenic potency. *J. Clin. Invest.* **2007**, *117* (9), 2562–2569.
(62) Giles, F. J.; Cortes, J.; Jones, D.; Bergstrom, D.; Kantarjian, H.; Freedman, S. J. MK-0457, a novel kinase inhibitor, is active in patients with chronic myeloid leukemia or acute lymphocytic leukemia with the T315I BCR-ABL mutation. *Blood* **2007**, *109* (2), 500–502.
(63) Gumireddy, K.; Baker, S. J.; Cosenza, S. C.; John, P.; Kang, A. D.; Robell, K. A.; Reddy, M. V. R.; Reddy, E. P. A non-ATP-competitive inhibitor of BCR-ABL overrides imatinib resistance. *Proc. Natl. Acad. Sci. U.S.A.* **2005**, *102* (6), 1992–1997.
(64) Miettinen, M.; Sarlomo-Rikala, M.; Lasota, J. Gastrointestinal stromal tumors: recent advances in understanding of their biology. *Hum. Pathol.* **1999**, *30* (10), 1213–1220.
(65) Hirota, S.; Isozaki, K.; Moriyama, Y.; Hashimoto, K.; Nishida, T.; Ishiguro, S.; Kawano, K.; Hanada, M.; Kurata, A.; Takeda, M.; Muhammad Tunio, G.; Matsuzawa, Y.; Kanakura, Y.; Shinomura, Y.; Kitamura, Y. Gain-of-function mutations of c-kit in human gastrointestinal stromal tumors. *Science* **1998**, *279* (5350), 577–580.
(66) Joensuu, H.; Roberts, P. J.; Sarlomo-Rikala, M.; Andersson, L. C.; Tervahartiala, P.; Tuveson, D.; Silberman, S. L.; Capdeville, R.; Dimitrijevic, S.; Druker, B.; Demetri, G. D. Effect of the tyrosine kinase inhibitor STI571 in a patient with a metastatic gastrointestinal stromal tumor. *N. Engl. J. Med.* **2001**, *344* (14), 1052–1056.
(67) Verweij, J.; Casali, P. G.; Zalcberg, J.; LeCesne, A.; Reichardt, P.; Blay, J.-Y.; Issels, R.; van Oosterom, A.; Hogendoorn, P. C. W.; Van Glabbeke, M.; Bertulli, R.; Judson, I. Progression-free survival in gastrointestinal stromal tumours with high-dose imatinib: randomised trial. *Lancet* **2004**, *364* (9440), 1127–1134.
(68) Demetri, G. D.; von Mehren, M.; Blanke, C. D.; Van den Abbeele, A. D.; Eisenberg, B.; Roberts, P. J.; Heinrich, M. C.; Tuveson, D. A.; Singer, S.; Janicek, M.; Fletcher, J. A.; Silverman, S. G.; Silberman, S. L.; Capdeville, R.; Kiese, B.; Peng, B.; Dimitrijevic, S.; Druker, B. J.; Corless, C.; Fletcher, C. D.; Joensuu, H. Efficacy and safety of imatinib mesylate in advanced gastrointestinal stromal tumors. *N. Engl. J. Med.* **2002**, *347* (7), 472–480. [see Comment].
(69) Mol, C. D.; Dougan, D. R.; Schneider, T. R.; Skene, R. J.; Kraus, M. L.; Scheibe, D. N.; Snell, G. P.; Zou, H.; Sang, B.-C.; Wilson, K. P. Structural basis for the autoinhibition and STI-571 inhibition of c-Kit tyrosine kinase. *J. Biol. Chem.* **2004**, *279* (30), 31655–31663.
(70) Antonescu, C. R.; Besmer, P.; Guo, T.; Arkun, K.; Hom, G.; Koryotowski, B.; Leversha, M. A.; Jeffrey, P. D.; Desantis, D.; Singer, S.; Brennan, M. F.; Maki, R. G.; DeMatteo, R. P. Acquired resistance to imatinib in gastrointestinal stromal tumor occurs through secondary gene mutation. *Clin. Cancer Res.* **2005**, *11* (11), 4182–4190.
(71) Chen, L. L.; Trent, J. C.; Wu, E. F.; Fuller, G. N.; Ramdas, L.; Zhang, W.; Raymond, A. K.; Prieto, V. G.; Oyedeji, C. O.; Hunt, K. K.; Pollock, R. E.; Feig, B. W.; Hayes, K. J.; Choi, H.; Macapinlac, H. A.; Hittelman, W.; Velasco, M. A.; Patel, S.; Burgess, M. A.; Benjamin, R. S.; Frazier, M. L. A missense mutation in KIT kinase domain 1 correlates with imatinib resistance in gastrointestinal stromal tumors. *Cancer Res.* **2004**, *64* (17), 5913–5919.
(72) Nishida, T.; Kanda, T.; Nishitani, A.; Takahashi, T.; Nakajima, K.; Ishikawa, T.; Hirota, S. Secondary mutations in the kinase domain of the KIT gene are predominant in imatinib-resistant gastrointestinal stromal tumor. *Cancer Sci.* **2008**, *99* (4), 799–804.
(73) Wardelmann, E.; Thomas, N.; Merkelbach-Bruse, S.; Pauls, K.; Speidel, N.; Büttner, R.; Bihl, H.; Leutner, C. C.; Heinicke, T.; Hohenberger, P. Acquired resistance to imatinib in gastrointestinal stromal tumours caused by multiple KIT mutations. *Lancet Oncol.*

2005, *6* (4), 249–251.
(74) Bickenbach, K.; Wilcox, R.; Veerapong, J.; Kindler, H. L.; Posner, M. C.; Noffsinger, A.; Roggin, K. K. A review of resistance patterns and phenotypic changes in gastrointestinal stromal tumors following imatinib mesylate therapy. *J. Gastrointest. Surg.* **2007**, *11* (6), 758–766.
(75) Guo, T.; Agaram, N. P.; Wong, G. C.; Hom, G.; D'Adamo, D.; Maki, R. G.; Schwartz, G. K.; Veach, D.; Clarkson, B. D.; Singer, S.; DeMatteo, R. P.; Besmer, P.; Antonescu, C. R. Sorafenib inhibits the imatinib-resistant KITT670I gatekeeper mutation in gastrointestinal stromal tumor. *Clin. Cancer Res.* **2007**, *13* (16), 4874–4881.
(76) Nagata, H.; Worobec, A. S.; Oh, C. K.; Chowdhury, B. A.; Tannenbaum, S.; Suzuki, Y.; Metcalfe, D. D. Identification of a point mutation in the catalytic domain of the protooncogene c-kit in peripheral blood mononuclear cells of patients who have mastocytosis with an associated hematologic disorder. *Proc. Natl. Acad. Sci. U.S.A.* **1995**, *92* (23), 10560–10564.
(77) Druker, B. J.; Tamura, S.; Buchdunger, E.; Ohno, S.; Segal, G. M.; Fanning, S.; Zimmermann, J.; Lydon, N. B. Effects of a selective inhibitor of the Abl tyrosine kinase on the growth of Bcr-Abl positive cells. *Nat. Med.* **1996**, *2* (5), 561–566.
(78) Buchdunger, E.; Cioffi, C. L.; Law, N.; Stover, D.; Ohno-Jones, S.; Druker, B. J.; Lydon, N. B. Abl protein−tyrosine kinase inhibitor STI571 inhibits in vitro signal transduction mediated by c-kit and platelet-derived growth factor receptors. *J. Pharmacol. Exp. Ther.* **2000**, *295* (1), 139–145.
(79) Shimizu, A.; O'Brien, K. P.; Sjoblom, T.; Pietras, K.; Buchdunger, E.; Collins, V. P.; Heldin, C.-H.; Dumanski, J. P.; Ostman, A. The dermatofibrosarcoma protuberans-associated collagen type Iα1/platelet-derived growth factor (PDGF) B-chain fusion gene generates a transforming protein that is processed to functional PDGF-BB. *Cancer Res.* **1999**, *59* (15), 3719–3723.
(80) Simon, M. P.; Pedeutour, F.; Sirvent, N.; Grosgeorge, J.; Minoletti, F.; Coindre, J. M.; Terrier-Lacombe, M. J.; Mandahl, N.; Craver, R. D.; Blin, N.; Sozzi, G.; Turc-Carel, C.; O'Brien, K. P.; Kedra, D.; Fransson, I.; Guilbaud, C.; Dumanski, J. P. Deregulation of the platelet-derived growth factor B-chain gene via fusion with collagen gene COL1A1 in dermatofibrosarcoma protuberans and giant-cell fibroblastoma. *Nat. Genet.* **1997**, *15* (1), 95–98.
(81) Sjoblom, T.; Boureux, A.; Ronnstrand, L.; Heldin, C. H.; Ghysdael, J.; Ostman, A. Characterization of the chronic myelomonocytic leukemia associated TEL-PDGF beta R fusion protein. *Oncogene* **1999**, *18* (50), 7055–7062.
(82) Jousset, C.; Carron, C.; Boureux, A.; Quang, C. T.; Oury, C.; Dusanter-Fourt, I.; Charon, M.; Levin, J.; Bernard, O.; Ghysdael, J. A domain of TEL conserved in a subset of ETS proteins defines a specific oligomerization interface essential to the mitogenic properties of the TEL-PDGFR beta oncoprotein. *EMBO J.* **1997**, *16* (1), 69–82.
(83) Apperley, J. F.; Gardembas, M.; Melo, J. V.; Russell-Jones, R.; Bain, B. J.; Baxter, E. J.; Chase, A.; Chessells, J. M.; Colombat, M.; Dearden, C. E.; Dimitrijevic, S.; Mahon, F. X.; Marin, D.; Nikolova, Z.; Olavarria, E.; Silberman, S.; Schultheis, B.; Cross, N. C.; Goldman, J. M. Response to imatinib mesylate in patients with chronic myeloproliferative diseases with rearrangements of the platelet-derived growth factor receptor beta. *N. Engl. J. Med.* **2002**, *347* (7), 481–487.
(84) Maki, R. G.; Awan, R. A.; Dixon, R. H.; Jhanwar, S.; Antonescu, C. R. Differential sensitivity to imatinib of 2 patients with metastatic sarcoma arising from dermatofibrosarcoma protuberans. *Int. J. Cancer* **2002**, *100* (6), 623–626.
(85) Rubin, B. P.; Schuetze, S. M.; Eary, J. F.; Norwood, T. H.; Mirza, S.; Conrad, E. U.; Bruckner, J. D. Molecular targeting of platelet-derived growth factor B by imatinib mesylate in a patient with metastatic dermatofibrosarcoma protuberans. *J. Clin. Oncol.* **2002**, *20* (17), 3586–3591. [see Comment].
(86) Baccarani, M.; Cilloni, D.; Rondoni, M.; Ottaviani, E.; Messa, F.; Merante, S.; Tiribelli, M.; Buccisano, F.; Testoni, N.; Gottardi, E.; de Vivo, A.; Giugliano, E.; Iacobucci, I.; Paolini, S.; Soverini, S.; Rosti, G.; Rancati, F.; Astolfi, C.; Pane, F.; Saglio, G.; Martinelli, G. The efficacy of imatinib mesylate in patients with FIP1L1-PDGFRalpha-positive hypereosinophilic syndrome. Results of a multicenter prospective study. *Haematologica* **2007**, *92* (9), 1173–1179. [see Comment].
(87) Jovanovic, J. V.; Score, J.; Waghorn, K.; Cilloni, D.; Gottardi, E.; Metzgeroth, G.; Erben, P.; Popp, H.; Walz, C.; Hochhaus, A.; Roche-Lestienne, C.; Preudhomme, C.; Solomon, E.; Apperley, J.; Rondoni, M.; Ottaviani, E.; Martinelli, G.; Brito-Babapulle, F.; Saglio, G.; Hehlmann, R.; Cross, N. C.; Reiter, A.; Grimwade, D. Low-dose imatinib mesylate leads to rapid induction of major molecular responses and achievement of complete molecular remission in FIP1L1-PDGFRA-positive chronic eosinophilic leukemia. *Blood* **2007**, *109* (11), 4635–4640.
(88) Antoniu, S. A. Imatinib mesylate for the treatment of hypereosinophilic syndromes. *Curr. Opin. Invest. Drugs* **2006**, *7* (11), 980–984.
(89) Cortes, J.; Ault, P.; Koller, C.; Thomas, D.; Ferrajoli, A.; Wierda, W.; Rios, M. B.; Letvak, L.; Kaled, E. S.; Kantarjian, H. Efficacy of imatinib mesylate in the treatment of idiopathic hypereosinophilic syndrome. *Blood* **2003**, *101* (12), 4714–4716.
(90) Pardanani, A.; Reeder, T.; Porrata, L. F.; Li, C. Y.; Tazelaar, H. D.; Baxter, E. J.; Witzig, T. E.; Cross, N. C.; Tefferi, A. Imatinib therapy for hypereosinophilic syndrome and other eosinophilic disorders. *Blood* **2003**, *101* (9), 3391–3397. [see Comment].
(91) Ault, P.; Cortes, J.; Koller, C.; Kaled, E. S.; Kantarjian, H. Response of idiopathic hypereosinophilic syndrome to treatment with imatinib mesylate. *Leukemia Res.* **2002**, *26* (9), 881–884.
(92) Gleich, G. J.; Leiferman, K. M.; Pardanani, A.; Tefferi, A.; Butterfield, J. H. Treatment of hypereosinophilic syndrome with imatinib mesilate. *Lancet* **2002**, *359* (9317), 1577–1578.
(93) Cools, J.; DeAngelo, D. J.; Gotlib, J.; Stover, E. H.; Legare, R. D.; Cortes, J.; Kutok, J.; Clark, J.; Galinsky, I.; Griffin, J. D.; Cross, N. C.; Tefferi, A.; Malone, J.; Alam, R.; Schrier, S. L.; Schmid, J.; Rose, M.; Vandenberghe, P.; Verhoef, G.; Boogaerts, M.; Wlodarska, I.; Kantarjian, H.; Marynen, P.; Coutre, S. E.; Stone, R.; Gilliland, D. G. A tyrosine kinase created by fusion of the PDGFRA and FIP1L1 genes as a therapeutic target of imatinib in idiopathic hypereosinophilic syndrome. *N. Engl. J. Med.* **2003**, *348* (13), 1201–1214. [see Comment].
(94) Hynes, N. E.; Lane, H. A. ERBB receptors and cancer: the complexity of targeted inhibitors. *Nat. Rev. Cancer* **2005**, *5* (5), 341–354.
(95) Dowell, J. E.; Minna, J. D. Chasing mutations in the epidermal growth factor in lung cancer. *New Engl. J. Med.* **2007**, *352*, 830–832.
(96) Pao, W.; Politi, K. A.; Reily, G. I.; Somwar, R.; Zakowski, M. F.; Kris, M. G.; Varmus, H. Acquired resistance of lung adenocarcinomas to gefitinib or erlotinib is associated with a second mutation in the EGFR kinase domain. *PLoS Med.* **2005**, *2* (3), e73.
(97) Kwak, E. L.; Sordella, R.; Bell, D. W.; Godin-Heymann, N.; Okimoto, R. A.; Brannigan, B. W.; Harris, P. L.; Driscoll, D. R.; Fidias, P.; Lynch, T. J.; Rabindran, S. K.; McGinnis, J. P.; Wissner, A.; Sharma, S. V.; Isselbacher, K. J.; Settleman, J.; Haber, D. A. Irreversible inhibitors of the EGF receptor may circumvent acquired resistance to gefitinib. *Proc. Nat. Acad. Sci. U.S.A.* **2005**, *102*, 7665–7670.
(98) Kumar, A.; Petri, E. T.; Halmos, B.; Boggon, T. J. Structure and clinical relevance fo the epidermal growth factor receptor in human cancer. *J. Clin. Oncol.* **2008**, *26* (10), 1742–1751.
(99) Stamos, J.; Sliwkowski, M. X.; Eigenbrot, C. Structure of the epidermal growth factor receptor kinase domain alone and in complex with a 4-anilinoquinazoline inhibitor. *J. Biol. Chem.* **2002**, *277* (48), 46265–46272.
(100) Michalczyk, A.; Kluter, S.; Rode, H. B.; Simard, J. R.; Grutter, C.; Baviller, M.; Rauh, D. Structural insights into how irreversible inhibitors can overcome drug resistance in EGFR. *Bioorg. Med. Chem.* **2008**, *16* (7), 3482–3488.
(101) Wissner, A.; Mansour, T. S. The development of HKI-272 and related compounds for the treatment of cancer. *Arch. Pharm. Chem. Life Sci.* **2008**, *341*, 465–477.
(102) Wong, K.-K. HKI-272 in non-small cell lung cancer. *Clin. Cancer Res.* **2007**, *13*, 4593–4596.
(103) Denny, W. A. Irreversible inhibitors of the erbB family of protein tyrosine kinases. *Pharmacol. Ther.* **2002**, *93*, 253–261.
(104) Bridges, A. J. The rationale and strategy used to develop a series of highly potent, irreversible, inhibitors of the epidermal growth factor receptor family of tyrosine kinases. *Curr. Top. Med. Chem.* **1999**, *6*, 825–843.
(105) Wissner, A.; Berger, D. M.; Boschelli, D. H.; Floyd, M. B. J.; Greenberger, L. M.; Gruber, B. C.; Johnson, B. D.; Mamuya, N.; Nilakantan, R.; Reich, M. F.; Shen, R.; Tsou, H.-R.; Upeslacis, E.; Wang, Y.-F.; Wu, B.; Ye, F.; Zhang, N. 4-Anilino-6,7-dialkoxyquinoline-3-carbonitrile inhibitors of epidermal growth factor receptor (EGF-R) kinase and their bioisosteric relationship to the 4-anilino-6,7-dialkoxyquinazoline inhibitors. *J. Med. Chem.* **2000**, *43*, 3244–3256.
(106) Li, D.; Ambrogio, L.; Shimamura, T.; Kubo, S.; Takahashi, M.; Chirieac, L. R.; Padera, R. G.; Shapiro, G. I.; Baum, A.; Himmelsbach, F.; Rettig, W. J.; Meyerson, M.; Solca, M.; Greulich, H.; Wong, K.-K. BIBW2992, an irreversible EGFR/HER2 inhibitor highly effective in preclinical lung cancer models. *Oncogene* **2008**, *27* (34), 4702–4711.
(107) Yun, C.-H.; Mengwasser, K. E.; Toms, A. V.; Woo, M. S.; Greulich, H.; Wong, K.-K.; Meyerson, M.; Eck, M. J. The T790M mutation in EGFR kinase causes drug resistance by increasing the affinity for ATP. *Proc. Natl. Acad. Sci. U.S.A.* **2008**, *105* (6), 2070–2075.
(108) Gray, N. S. Chemical Approaches for Making "Inactive-Conformation" Kinase Inhibitors. Presented at the 236th National Meeting of the American Chemical Society, Philadelphia, PA, 2008.

(109) Gajiwala, K. S.; Wu, J.; Lunney, E.; Demetri, G. Novel Mechanisms of Drug Resistance in KIT Mutants from Patients with Gastrointestinal Stromal Tumors: Structural Biology of Wild-Type and Mutated Proteins. Presented at the 236th National Meeting of the American Chemical Society, Philadelphia, PA, 2008.

(110) Zunder, E. R.; Knight, Z. A.; Houseman, B. T.; Apsel, B.; Shokat, K. M. Discovery of drug-resistant and drug-sensitizing mutations in the oncogenic PI3K isoform p110alpha. *Cancer Cell* **2008**, *14*, 180–192.

(111) Azam, M.; Latek, R. R.; Daley, G. Q. Mechanisms of autoinhibition and STI-571/imatinib resistance revealed by mutagenesis of BCR_ABL. *Cell* **2003**, *112*, 831–843.

(112) von Bubnoff, N.; Veach, D. R.; van der Kuip, H.; Aulitzky, W. E.; Sander, J.; Seipel, P.; Bornmann, W. G.; Peschel, C.; Clarkson, B.; Duyster, J. A cell-based screeen for resistance of Bcr-Abl-positive leukemia identieifs the mutation pattern for PD166326, an alternative Abl kinase inhibitor. *Blood* **2005**, *105*, 1652–1659.

(113) Girdler, F.; Sessa, F.; Patercoli, S.; Villa, F.; Musacchoi, A.; Taylor, S. Molecular basis of drug resistance in Aurora kinases. *Chem. Biol.* **2008**, *15* (6), 552–562.

(114) Wan, P. T. C.; Garnett, M. J.; Roe, S. M.; Lee, S.; Niculescu-Duvaz, D.; Good, V. M.; Project, C. G.; Jones, C. M.; Marshall, C. J.; Springer, C. J.; Barford, D.; Marais, R. Mechanism of activation of the RAF-ERK signaling pathway by oncogenic mutations of b-Raf. *Cell* **2004**, *116*, 855–867.

(115) Davies, H.; Bignell, G. R.; Cox, C.; Stephens, P.; Edkins, S.; Clegg, S.; Teague, J.; Woffendin, H.; Garnett, M. J.; Bottomley, W.; Davis, N.; Dicks, E.; Ewing, R.; Floyd, Y.; Gray, K.; Hall, S.; Hawes, R.; Hughes, J.; Kosmidou, V.; Menzies, A.; Mould, C.; Parker, A.; Stevens, C.; Watt, S.; Hooper, S.; Wilson, R.; Jayatilake, H.; Gusterson, B. A.; Cooper, C.; Shipley, J.; Hargrave, D.; Pritchard-Jones, K.; Mailtland, N.; Chenevix-Trench, G.; Riggins, G. J.; Bigner, D. D.; Palmieri, G.; Cossu, A.; Flanagan, A.; Nicholson, A.; Ho, J. W. C.; Leung, S. Y.; Yuen, S. T.; Weber, B. L.; Seigler, H. F.; Darrow, T. L.; Paterson, H.; Marais, R.; Marshall, C. J.; Wooster, R.; Stratton, M. R.; Futreal, P. A. Mutations of the BRAF gene in human cancer. *Nature* **2002**, *417*, 949–954.

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