STRATEGIES TO OVERCOME RESISTANCE TO TARGETED PROTEIN KINASE INHIBITORS

Henrik Daub, Katja Specht‡ and Axel Ullrich‡,§*

Abstract | Selective inhibition of protein tyrosine kinases is gaining importance as an effective therapeutic approach for the treatment of a wide range of human cancers. However, as extensively documented for the *BCR–ABL* oncogene in imatinib-treated leukaemia patients, clinical resistance caused by mutations in the targeted oncogene has been observed. Here, we look at how structural and mechanistic insights from imatinib-insensitive Bcr–Abl have been exploited to identify second-generation drugs that override acquired target resistance. These insights have created a rationale for the development of either multi-targeted protein kinase inhibitors or cocktails of selective antagonists as antitumour drugs that combine increased therapeutic potency with a reduced risk of the emergence of molecular resistance.

CHRONIC MYELOID LEUKAEMIA (CML). A myeloproliferative disorder that is characterized by a distinctive cytogenetic abnormality, the Philadelphia (Ph) chromosome.

**Axxima Pharmaceuticals AG, Max-Lebsche-Platz 32, 81377 München, Germany.*
*‡Centre for Molecular Medicine, Agency for Science, Technology and Research (A*STAR), 61 Biopolis Drive, Proteos, Singapore 138673.*
§Department of Molecular Biology, Max-Planck-Institute of Biochemistry, Am Klopferspitz 18A, 82152 Martinsried, Germany.
Correspondence to H.D.
e-mail: henrik.daub@axxima.com
doi:10.1038/nrd1579

Members of the protein kinase superfamily of enzymes regulate key aspects of human neoplasia such as tumour cell proliferation, migration and survival[1]. Deregulated protein kinase activity can result from genetic alterations acquired early in tumorigenesis and remains an essential aspect of tumour cell physiology throughout disease progression[2]. The targeted inhibition of protein kinases has therefore emerged as an attractive strategy in cancer treatment[3–5], and promises improved efficacy without the toxic side effects of currently used anticancer drugs.

The targeted inactivation of disease-relevant protein kinases is often pursued with ATP-competitive small-molecule inhibitors that block the enzymatic activity of kinases and thereby interfere with phosphorylation of cellular substrates. However, the therapeutic inactivation of an essential protein creates selective pressures, analagous to those in ordinary natural selection, for tumour cells to evolve mechanisms of resistance. In a manner similar to the widespread and extensively studied emergence of resistance formation in bacteria after exposure to antimicrobial agents[6,7], tumour cells can take a variety of routes to resistance. These include producing a drug-resistant variant of the targeted protein, substituting its cellular function by upregulating alternate pathways, and increasing the expression and function of transporters involved in drug efflux. Recent clinical data indicate that the emergence of drug-resistant kinase alleles is particularly relevant to anticancer therapy. Several recent reviews have dealt with this topic[8–12]. In this article, we review the structural aspects of drug resistance to protein kinases and discuss countermeasures to address this emerging problem that besets targeted cancer therapy.

Imatinib: lessons from a prototype inhibitor

The phenylaminopyrimidine compound imatinib mesylate (Gleevec/Glivec/STI571; Novartis) was among the first selective protein kinase inhibitors developed for targeted cancer therapy, and is highly effective against several tyrosine kinases such as Abl, Kit and the platelet-derived growth factor receptor (PDGFR)[13]. Treatment with imatinib as a single agent has demonstrated remarkable clinical efficacy in human malignancies such as CHRONIC MYELOID LEUKAEMIA (CML). In about 95% of all cases, CML pathogenesis is a consequence of a defined translocation event that creates the Philadelphia (Ph) chromosome, which results in the formation of the breakpoint cluster region–abelson tyrosine kinase (*BCR–ABL*) oncogene[14,15]. The deregulated, constitutive

Abl tyrosine kinase activity of the encoded Bcr–Abl fusion protein is crucial to malignant transformation and therefore represents an attractive target for therapeutic intervention[16–19]. In addition to CML, deregulated Abl kinase activity resulting from the Ph chromosomal translocation is also detected in up to 20% of adult acute lymphoblastic lymphoma (ALL) patients[20]. Durable haematological and cytogenetic responses to imatinib treatment were associated with progression-free survival in most cases of chronic-phase CML, whereas CML patients in the advanced disease states, such as BLAST CRISIS, or patients with Philadelphia-chromosome-positive (Ph$^+$) ALL, were either refractory to the drug or relapsed within several months despite continued imatinib therapy[21]. Observations of acquired resistance to imatinib have fuelled substantial research efforts during recent years to explore the causative molecular mechanisms underlying resistance. Most importantly, the formation of resistance to imatinib as a first-in-class, targeted, kinase-selective anticancer drug could represent an inherent problem of this therapeutic approach in general.

Basis of CML resistance to imatinib

Drug resistance in Ph$^+$ leukaemia has been attributed to a variety of different mechanisms (FIG. 1)[22]. Reduced drug delivery due to extracellular sequestration of imatinib by α1 acid glycoprotein in the plasma and P-glycoprotein-mediated active transport of imatinib out of the target cells have been proposed as processes that prevent imatinib from reaching its intracellular target, Bcr–Abl[23–25]. However, it is not yet clear whether these mechanisms contribute to imatinib resistance in relapsed leukaemia patients.

Another resistance mechanism could be the compensation of loss of Bcr–Abl signalling by other kinase pathways involving Src-family kinases such as Lyn, which was found to be upregulated in cultured CML cells selected for imatinib resistance[26,27]. Increased Lyn expression was also found to correlate with the formation of imatinib resistance in some patients, which highlights the potential clinical relevance of this resistance mechanism[26]. Alternatively, the actual expression of *BCR–ABL* itself can be upregulated to overcome the effect of imatinib, indicating an ongoing cellular dependence on Bcr–Abl activity. This has been shown in a subset of relapsed CML patients, whose levels of the Bcr–Abl fusion protein were elevated as a result of gene amplification[28]. In addition, despite its impressive therapeutic efficacy in halting disease progression in the early stages of CML, imatinib treatment does not seem to eradicate the actual cause of the disease — quiescent Ph$^+$ stem cells. In contrast to the mature, differentiated leukaemia cells, quiescent Ph$^+$ stem-cell progenitors seem to tolerate the inhibition of Bcr–Abl kinase activity and are therefore not forced into apoptosis on exposure to imatinib[29]. Subsequent proliferation of stem-cell clones is therefore likely to result in the reappearance of leukaemia on cessation of imatinib therapy. Although this does not account for a resistance mechanism per se, it nevertheless necessitates a life-long continuation of imatinib therapy.

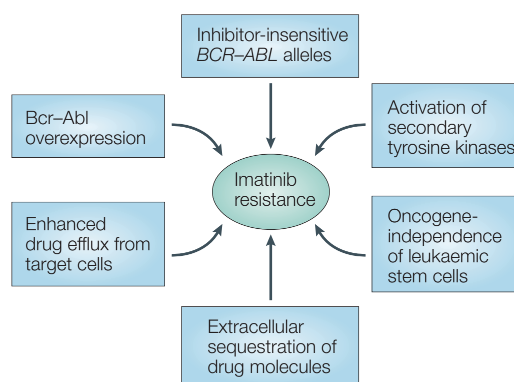

Figure 1 | **Potential mechanisms of resistance to imatinib in Philadelphia-chromosome-positive leukaemia.** In the majority of cases, resistance is caused by reactivation of Bcr–Abl kinase activity. Mutations in the *BCR–ABL* gene, predominantly in the kinase domain, are the most common mechanism implicated in secondary drug resistance. In some treated patients, *BCR–ABL* gene amplification and increased Bcr–Abl protein expression have been observed as a compensatory mechanism for the imatinib antitumour effect. The switch to alternative kinase signalling pathways, which can compensate for the loss of Bcr–Abl activity, has been proposed as another resistance strategy of leukaemic cells. Imatinib might also have different effects on chronic myelogenous leukaemia (CML) tumour cells depending on their differentiation state, and it has been proposed that quiescent CML stem cells might be intrinsically resistant to the drug. Other mechanisms of resistance to imatinib might be related to pharmacokinetic factors of drug delivery. Imatinib can be actively transported out of tumour cells through efflux pump proteins to keep intracellular drug concentrations below inhibitory levels. Extracellular sequestration of imatinib by α1 acid glycoprotein in the plasma has been proposed as a potential mechanism, which would result in reduced availability of the free drug to CML cells.

For the vast majority of relapsed leukaemia patients, however, resistance to imatinib is associated with the emergence of point mutations in the *BCR–ABL*-encoded kinase domain[28,30–35]. Numerous mutations within this domain have been found so far, and for many of these, biochemical and cellular assays have verified the amino-acid substitutions as molecular determinants for imatinib resistance of Bcr–Abl kinase activity. In the following part of this review, we focus on the imatinib-desensitizing mechanisms associated with the most prevalent *BCR–ABL* alleles detected in relapsed leukaemia patients.

Determinants of Bcr–Abl kinase resistance

Imatinib resistance of Bcr–Abl frequently results from mutations within amino-acid sequences that encode important structural features of the kinase, such as the 'gatekeeper' residue, the p-loop and the activation loop. The structure of the Abl kinase domain showing the location of these mutational hotspots is shown in FIG. 2, and comprehensive lists of known detected Abl variants have been compiled in recent review articles[9,10,12].

On binding, the hydroxyl group of Thr315 of Bcr–Abl, the so-called 'gatekeeper' residue, forms a hydrogen bond with imatinib, and the side chain present at position 315 also sterically controls the binding of the

BCR–ABL
The fusion gene that results from the chromosomal translocation that causes the Abelson protein tyrosine kinase gene to fuse with the BCR gene on the so-called Philadelphia (Ph) chromosome.

BLAST CRISIS
The aggressive phase of chronic myelogenous leukaemia evidenced by an increased number of immature white blood cells in the circulating blood.

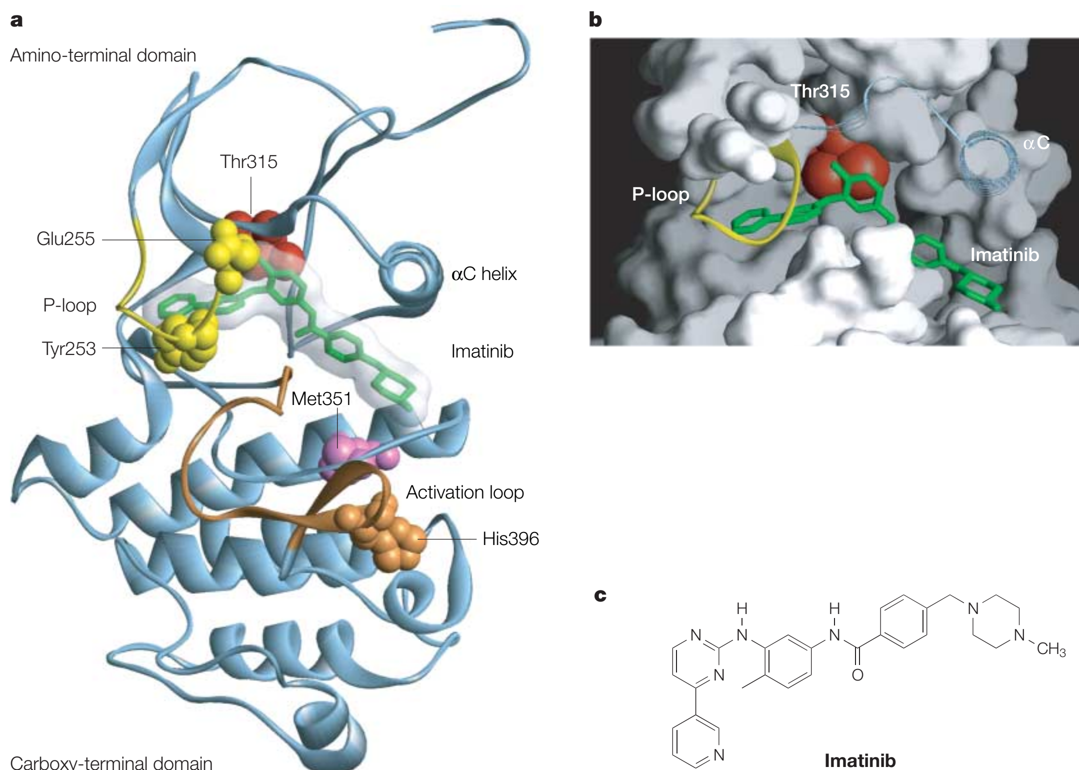

Figure 2 | **Mutational hotspots conferring imatinib resistance to Bcr–Abl. a** | The structure of the Abl kinase domain in complex with imatinib (green) is shown. Thr315, which is in direct contact with bound imatinib, is highlighted in red. The p-loop and the amino acids Tyr253 and Glu255, which are affected by frequently observed gene mutations in this region, are shown in yellow. The activation loop harbouring His396 is in orange and the residue Met351, which is affected by the clinically common M351T mutation, is highlighted in pink. **b** | A closer look at the imatinib binding site in Abl, most of which is shown as surface representation, including the gatekeeper Thr315 highlighted in red. From this perspective, the p-loop would cover the underlying imatinib contact regions and is therefore displayed as a ribbon. **c** | The chemical structure of imatinib.

inhibitor to hydrophobic regions adjacent to the ATP-binding site (FIG. 2). One of the most frequently detected mutations in imatinib-resistant CML is a T315I substitution, which directly interferes with imatinib binding because of the introduction of a large isoleucine side chain into the gatekeeper position[28,36]. Importantly, although the gatekeeper threonine is essential for imatinib, this is not the case for ATP binding, which does not depend on the accessibility of the same hydrophobic cavity and is therefore not affected by the incorporation of a bulky isoleucine side chain. The individual structural requirements of ATP and imatinib mean that the catalytic activity, and therefore the tumour-promoting function, is preserved in the imatinib-insensitive T315I mutant of Bcr–Abl. Gatekeeper amino-acid mutations affect the sensitivities of various protein kinase targets for diverse drug chemotypes and so this structural determinant has general relevance to kinase inhibitor development, and will be discussed in more detail in the following sections[37].

Imatinib can be distinguished from most kinase inhibitors by another structural requirement for its binding. Co-crystal structure analysis revealed that imatinib selectively binds to a distorted inactive conformation of the Abl kinase domain through an INDUCED-FIT MECHANISM[9,36,38]. Remarkably, the potency of imatinib towards constitutively active Bcr–Abl implies a dynamic equilibrium from which imatinib can trap the deregulated Bcr–Abl oncoprotein when it transits through its inactive conformation. This mode of binding is potentially an Achilles' heel for imatinib compared with other small-molecule kinase inhibitors that are effective against the catalytically active kinase conformation. Indeed, *BCR–ABL* sequence analysis in relapsed CML and Ph$^+$ ALL patients revealed the clustering of imatinib-desensitizing mutations in regions of the kinase domain, where structural alterations occur on imatinib binding[33]. Mutations were frequently detected at positions such as Tyr253 and Glu255 located in the previously mentioned p-loop (FIG. 2). These amino-acid substitutions seem to interfere with the distorted p-loop conformation that is crucial for imatinib binding and, consistent with the observed *in vivo* resistance and the particularly poor prognosis of patients affected by p-loop mutations such as Y253F and E255K[39], strongly reduced the potency of imatinib in inhibiting Bcr–Abl activity in biochemical and cellular assays.

A second structural requirement for imatinib binding is defined by the inactive, unphosphorylated state of the activation loop. Mutations in this region, such as H396R, destabilize an Abl-specific, closed conformation of the activation loop and thereby counteract imatinib

INDUCED-FIT MECHANISM
The interaction between a protein and ligand in which the binding of the ligand alters the conformation of the protein's active site to best accommodate binding of the ligand.

inhibition (FIG. 2)[33,35]. In addition, molecular studies on the formation of clinical resistance revealed a group of point mutations remote from the imatinib binding site, and which lie in the carboxy-terminal lobe of the kinase domain. The most frequently detected Abl variant falling into this group is a M351T mutation and accounts for 15–20% of all cases of observed clinical resistance[10]. It would seem that the M351T mutation affects the precise positioning of residues in direct contact with imatinib[9,33], although these small structural alterations are difficult to predict because Met351 is rather remote from the imatinib-binding site (FIG. 2).

The mutations focused on in this review are restricted to the kinase domain of Bcr–Abl. However, as indicated by a saturating mutational analysis of full-length *BCR–ABL* combined with a cellular screening procedure selecting for Bcr–Abl-driven cell proliferation in the presence of imatinib, mutations outside of the kinase domain can weaken the interaction with imatinib and thereby contribute to target resistance[40]. These mutations would probably have escaped detection in all earlier clinical studies because sequence analysis has mainly been restricted to the Abl kinase domain.

Imatinib resistance is not specific for Bcr–Abl

The Ph chromosomal translocation that generates the *BCR–ABL* oncogene represents the most common cytogenetic abnormality linked to myeloproliferative disease. However, gene rearrangements are not exclusive for CML and Ph$^+$ ALL. Similar molecular mechanisms create imatinib-sensitive PDGFRα and PDGFRβ fusion proteins implicated in rare haematologic diseases such as IDIOPATHIC HYPEREOSINOPHILIC SYNDROME and chronic myelomonocytic leukaemia, respectively[41,42]. In hypereosinophilic syndrome, excessive proliferation of eosinophils often results from the constitutive tyrosine kinase activity of the chimeric Fip1-like 1 (FIP1L1)–PDGFRα protein, which apparently arises from the fusion of the *FIP1L1* and *PDGFRA* genes by an INTERSTITIAL CHROMOSOMAL DELETION[42]. FIP1L1–PDGFRα-induced transformation of haematopoietic cells was highly sensitive to imatinib inhibition *in vitro*, occurring at about 100-fold lower drug concentration than required to suppress Bcr–Abl-induced cell proliferation[42]. In a study by Cools *et al.*, five out of five imatinib-treated patients with a detectable *FIP1L1–PDGFRα* gene fusion experienced complete haematological remission, including a return of the eosinophil count to normal values[42]. However, one of these patients eventually relapsed and was found to be resistant to imatinib, which was attributed to a threonine to isoleucine substitution in the ATP-binding site of PDGFRα kinase at an equivalent position to the gatekeeper Thr315 of Abl (FIG. 3a).

Imatinib monotherapy has also demonstrated remarkable efficacy against gastrointestinal stromal tumours (GIST), which often harbour activating mutations in the juxtamembrane domain of the imatinib-sensitive Kit receptor tyrosine kinase[43]. Secondary resistance formation to imatinib was recently reported for a GIST patient and was caused by a T670I mutation in the Kit kinase domain[44], which is again in a homologous position to the gatekeeper residue of Abl (FIG. 3a). The mutation was confined to a metastatic lesion of the solid tumour, which progressed during continued imatinib therapy while other lesions were still responding to imatinib treatment[44].

Together, these clinical results indicate at least one common mechanism of resistance formation shared by the imatinib targets Abl, PDGFRα and Kit. The development of resistance to imatinib is therefore not exclusively a consequence of the increased genomic instability associated with Bcr–Abl expression, but might actually be a more widespread drawback to imatinib monotherapy. By contrast, however, the mutational hotspots Tyr253 and Glu255, which are located in the p-loop of Abl, are not conserved in PDGFR and Kit (FIG. 3b). In fact, the wild-type alleles of both receptor tyrosine kinases already possess phenylalanine and lysine residues homologous to the mutated amino acids often detected in the imatinib-resistant Y253F and E255K variants of Abl[9,10,12]. Therefore, in contrast to the mutation of the common threonine gatekeeper residue that directly interacts with the bound drug and is observed in all three tyrosine kinases, mutations in the p-loop of Abl, which destabilize the imatinib-specific inactive conformation, would not occur in PDGFR and Kit, according to primary sequence alignments of the different imatinib targets.

It is significant to note, however, the recent emergence of a secondary *KIT* mutation during imatinib therapy, in which the Tyr823 residue of Kit, which is homologous to the major phosphorylation site Tyr393 within the activation loop of Abl, was substituted with aspartate[45]. Phosphorylation of this site in wild-type Abl desensitizes the kinase to inhibition by imatinib by destabilizing the inactive conformation of the activation loop[36]. A negatively charged aspartate side chain at the corresponding position of Kit might therefore mimic this phosphorylated state and result in similar resistance. Moreover, another activation loop mutation has been characterized for Kit, albeit not in the context of relapse from imatinib therapy. The kinase-activating D816V mutation in the *KIT* proto-oncogene confers primary imatinib resistance to the receptor tyrosine kinase (RTK) and has been identified as a genetic cause of disease in human mastocytosis[46] (FIG. 3c). This substitution, which is not conserved in Abl, might also prevent Kit from adopting the distorted conformation required for imatinib binding.

To evaluate the potential modes of resistance acquisition to imatinib in the absence of further clinical data, mutagenesis of oncogenic *PDGFR* and *KIT* variants in combination with cellular *in vitro* screens for imatinib resistance could be performed with the same experimental strategy as reported for *BCR–ABL*[40]. This type of approach could anticipate mechanisms of imatinib resistance before they become a clinical reality, and therefore prompt the testing of potential back-up drugs against tyrosine kinase variants that are likely to be seen during relapse of disease.

IDIOPATHIC HYPEREOSINOPHILIC SYNDROME
The presence of prolonged eosinophilia without an identifiable underlying cause and with evidence of end-organ dysfunction.

INTERSTITIAL CHROMOSOMAL DELETION
Loss of material from within one of the chromosome arms.

a

Abl	E	P	P	F	Y	I	I	T_{315}	E	F	M	T	Y	G	N
PDGFRα	S	G	P	I	Y	I	I	T_{674}	E	Y	C	F	Y	G	D
Kit	G	G	P	T	L	V	I	T_{670}	E	Y	C	C	Y	G	D

b

Abl	G	G	G	Q	Y_{253}	G	E_{255}	V	Y	E
PDGFRα	G	S	G	A	F	G	K	V	V	E
Kit	G	A	G	A	F	G	K	V	V	E

c

Abl	L	S	R	L	M	T	G	D	T	–	Y	T	A	H_{396}	A	G
PDGFRα	L	A	R	D	I	M	H	D	S	N	Y	V	S	K	G	S
Kit	L	A	R	D_{816}	I	K	N	D	S	N	Y_{823}	V	V	K	G	N

Figure 3 | **Sequence alignments of the imatinib targets Abl, PDGFRα and Kit. a** | Residues surrounding the conserved gatekeeper threonine at the ATP-binding site of the tyrosine kinases. Mutation of this residue to isoleucine accounts for a common mechanism of clinical resistance to imatinib. **b** | Alignment of the p-loop sequences. The frequently mutated Abl residues Tyr253 and Glu255 are not conserved in the platelet-derived growth factor receptor-α (PDGFRα) and Kit. Remarkably, the Y253F and E255K alleles of *BCR–ABL* are frequently detected in the context of clinical resistance and imatinib-sensitive PDGFRα and Kit already possess the phenylalanine and lysine residues that confer imatinib resistance to Abl tyrosine kinase at the equivalent positions, indicating that desensitizing p-loop mutations are not conserved among different imatinib targets. **c** | Alignment of a stretch of amino acids, which includes parts of the activation loop. Mutation of Asp816 desensitizes Kit to imatinib inhibition. This residue is not conserved in Abl. Conversely, the mutational site His396 of Abl is not conserved in the two receptor tyrosine kinases. Mutation of Tyr823 of Kit was implicated in clinical imatinib resistance. Despite the conservation of this site in Abl, clinical *BCR–ABL* variants with mutations at this position have not emerged during imatinib therapy.

Second-generation Abl kinase inhibitors

The emergence of drug-resistant *BCR–ABL* alleles during imatinib therapy provides genetic evidence for the ongoing requirement of cellular Bcr–Abl kinase activity in disease progression and reconfirms that mutated variants are still viable targets for therapeutic intervention. Most clinical Bcr–Abl mutations distort the inactive Abl conformation required for imatinib binding, and this could therefore be circumvented by developing drugs that target the active configuration of the kinase.

The catalytically active state of Abl closely resembles the active form of Src-family kinases and is often potently inhibited by ATP-competitive inhibitors originally identified as Src antagonists (FIG. 4a)[38,47]. Testing of dual-specificity Abl- and Src-family kinase inhibitors belonging to the pyrido[2,3-*d*]pyrimidine class of compounds revealed considerable inhibitory activity against most clinically relevant Bcr–Abl isoforms except the T315I variant, indicating that the gatekeeper residue also sterically controls the binding of pyrido[2,3-*d*]pyrimidine inhibitors[48–50].

Pyrido[2,3-*d*]pyrimidines such as PD180970 (FIG. 4a) have been shown to suppress the cancer-promoting activity of activation loop mutants such as H396P at similar concentrations as wild-type Bcr–Abl, whereas the IC_{50} for imatinib inhibition of the same mutant was about tenfold higher[48,50]. These compounds also potently inhibit the clinically common p-loop mutations that affect Tyr253 or Glu255 in the glycine-rich region of Abl, although this inhibition requires concentrations approximately fivefold higher than those required to inhibit non-mutated Bcr–Abl. However, the p-loop mutants were still more sensitive to the pyrido[2,3-*d*]pyrimidines than to imatinib, which correlates with the poor prognosis associated with these imatinib-resistant mutants in the clinic[39]. A slightly increased concentration of PD180970 inactivated the frequently detected M351T mutant, which accounts for about 15–20% of imatinib-resistant CML cases, compared with the concentration required to block wild-type Bcr–Abl[48]. However, this variant is only moderately resistant to imatinib and requires three- to fivefold higher imatinib concentrations to obtain half-maximal inhibition of Bcr–Abl in intact cells and might therefore be amenable to dose escalation in leukaemia patients[51].

The promising results with pyrido[2,3-*d*]pyrimidines generated interest in structurally different inhibitors of Src and Abl tyrosine kinases, including the compounds BMS-354825 and AP23464 (FIG. 4b,c)[52,53]. These back-up drugs were quite similar to pyrido[2,3-*d*]pyrimidines in their abilities to inhibit the clinically common Bcr–Abl alleles that cause imatinib resistance. BMS-354825 also demonstrated *in vivo* activity against the M351T allele in a mouse model of CML[52]. However, it could be that increased imatinib doses would have a similar result on the M351T variant in animal experiments.

Although none of the drugs were effective against the Abl gatekeeper-residue mutant T315I, their potency against other imatinib-resistant Bcr–Abl proteins further indicates that imatinib is particularly sensitive to resistance because of the stringent conformational requirements for its binding. However, the targeting of the inactive kinase conformation that is characteristic of imatinib is likely to result in higher selectivity compared with back-up drugs directed against the relatively conserved active state[36,38]. Less selectivity might lead to more adverse side effects in leukaemia patients, and so it remains to be determined whether second-generation drugs with favourable clinical toxicity profiles can be developed against imatinib-resistant alleles of Bcr–Abl.

Targeting the gatekeeper

Neither imatinib nor any of the described second-generation inhibitors are currently effective against the T315I allele of Bcr–Abl. It is therefore of concern, when considering the efficacy of these drug classes, that the corresponding threonine residue — or a valine side chain of similar size, which is found in a subset of all human protein kinases — might represent a conserved hotspot for resistance formation[37,54]. For example, mutation of the gatekeeper residue to larger residues, such as isoleucine or methionine, confers resistance against various kinases such as p38, Src and the epidermal growth factor receptor (EGFR) against different types of ATP-competitive inhibitors, including SB203580, PP1 and PD153035, respectively[55–57]. All of these inhibitors possess aromatic substituents, which are accommodated by the hydrophobic pocket that is adjacent to the ATP-binding site in the wild-type kinase. As illustrated

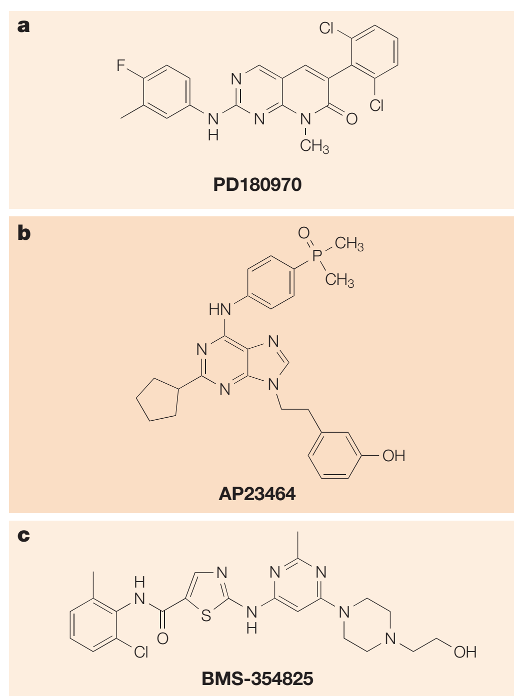

Figure 4 | **Chemical structures of the imatinib back-up drugs.** PD180970 (**a**), AP23464 (**b**) and BMS-354825 (**c**) have been characterized as potent dual-specificity inhibitors of Abl- and Src-family kinases. In contrast to imatinib, PD180970, BMS-354825 and AP23464 target the active configuration of Abl tyrosine kinase and therefore retain considerable inhibitory activity against clinical *BCR–ABL* variants, which destabilize the inactive conformation required for efficient imatinib binding.

by the crystal structure of the EGFR in complex with the PD153035-related inhibitor erlotinib (FIG. 5a,b), larger residues in the position of the crucial Thr766 would sterically interfere with the projection of the inhibitor into that cavity and thereby induce target resistance[58]. As previously mentioned, the hydrophobic pocket is not involved in ATP binding (FIG. 5c), which is why catalytic activity is preserved in kinase variants with mutations at this site.

Gefitinib (Iressa/ZD1839; AstraZeneca), an EGFR inhibitor, was recently approved for the treatment of advanced non-small-cell lung cancers (NSCLCs) that do not respond to established chemotherapy regimens[59,60]. The C-to-T single-nucleotide mutation that leads to the imatinib-refractory T351I allele of *BCR–ABL* also dramatically desensitizes EGFR to gefitinib by replacing the corresponding gatekeeper residue Thr766 with a methionine residue[37,57]. In light of several recent studies that have established a strong correlation between objective tumour responses to the drug and kinase-activating mutations in the EGFR found in a relatively small subset of NSCLC patients[61–64], there is perhaps cause for concern about the potential for resistance formation against gefitinib due to mutation of the essential gatekeeper residue.

HUMAN KINOME
The collection of genes in the human genome encoding kinases.

One way to override the resistance caused by mutation of a gatekeeper residue would be to develop small-molecule compounds that do not depend on this structural element for binding. This has been demonstrated for the imatinib-insensitive T674I variant of the constitutively active FIP1L1–PDGFRα fusion protein implicated in hypereosinophilic syndrome, which was effectively inhibited by the staurosporine analogue PKC412 both *in vitro* and in a mouse model of myeloproliferative disease[65]. The corresponding T681I mutation in the closely related PDGFRβ tyrosine kinase did not confer resistance to the indolinone compound SU6668, which was in contrast to the target desensitization observed for several other PDGFR inhibitors, including imatinib[37]. Moreover, related inhibitors based on the same scaffold as SU6668 were effective against fibroblast growth factor receptor 1 (FGFR1) and Src tyrosine kinase mutants harbouring a methionine in the gatekeeper position[37,66]. Consistent with these findings, structural analysis revealed that these compounds do not bind in the hydrophobic back pocket adjacent to the nucleotide-binding site.

These molecular insights are not only relevant in the context of acquired drug resistance, but also relate to important aspects of compound library design and composition for screening purposes against protein kinase targets. About 75% of all human protein kinases have larger, hydrophobic residues such as methionine, leucine or phenylalanine in the position homologous to Thr315 of Abl and Thr766 of EGFR[54]. This large part of the HUMAN KINOME also includes various oncology targets such as cyclin-dependent kinases, insulin-like growth factor 1 receptor or FLT3, which can be expected to be rather insensitive to small-molecule inhibitors that extend bulky, aromatic substituents accommodated into the hydrophobic back pocket.

Multi-targeted therapy with kinase inhibitors

Obviously, any anticancer drug sets up a selective pressure for those tumour cells that can survive and proliferate in its presence. The same basic principle seems to be true for protein kinase inhibitors, as exemplified by the clonal selection for drug-resistant *BCR–ABL* alleles during imatinib therapy. A general drawback of target-specific monotherapy therefore derives from the fact that a single genetic alteration conferring target resistance to an individual tumour cell can eventually lead to relapse. Once second-generation drugs targeting imatinib-resistant Bcr–Abl become available, their clinical use could be guided by sensitive diagnostic tools such as allele-specific oligonucleotide polymerase chain reaction (ASO-PCR), which can be used to predict therapeutic efficacy in individual patients on the basis of the type of the detected mutations[67]. Molecular characterization of *BCR–ABL* alleles might also be considered early in therapy of late-stage CML or Ph$^+$ ALL patients, because in some cases *BCR–ABL* mutations have even been found to exist in a small subpopulation of leukaemic cells before imatinib treatment[67–69]. These approaches will probably find applications in tailoring the use of other targeted kinase inhibitors, such as gefitinib.

However, the ability of kinases to mutate in response to the selective pressure created by drug treatment

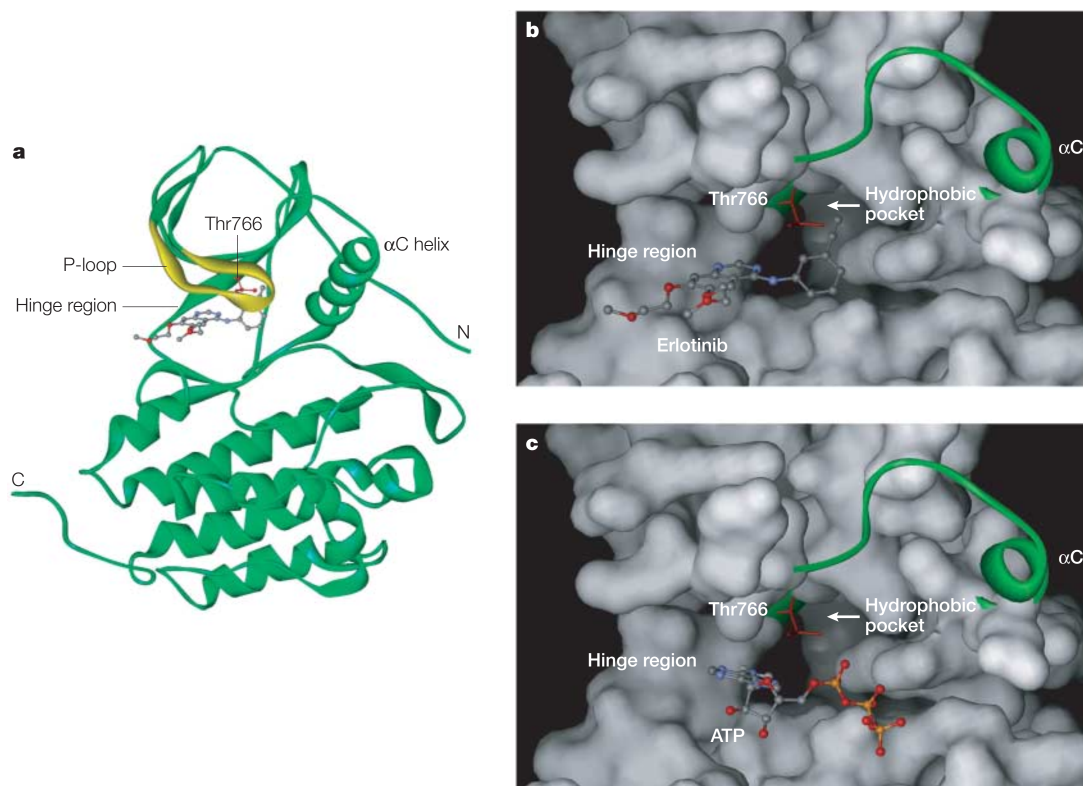

Figure 5 | **Relevance of the gatekeeper residue for inhibitor binding. a** | Ribbon representation of the structure of the epidermal growth factor receptor (EGFR) tyrosine kinase domain in complex with the ATP-competitive inhibitor erlotinib[58], which is a small-molecule antagonist of EGFR tyrosine kinase and belongs to the same class of compounds as gefitinib. The p-loop is highlighted in yellow. The position of the αC helix, which represents a conserved structural feature in the amino-lobe of protein kinase domain, is shown for reference. **b** | Orientation of erlotinib in the nucleotide-binding pocket of the EGFR. The p-loop is not shown in this presentation. The positions of the hinge region, the hydrophobic back pocket and the gatekeeper threonine residue (Thr766) are indicated. Substitution of the Thr766 by a larger residue would result in a steric clash with the aromatic moiety of the inhibitor accommodated by the hydrophobic pocket. **c** | Insertion of ATP into the nucleotide-binding site of the EGFR crystal structure. ATP does not interact with the hydrophobic region involved in erlotinib binding, illustrating why larger residues in the gatekeeper position 766 do not abrogate the catalytic activity of the EGFR tyrosine kinase[35,57].

provides a strong rationale for hitting more than one essential target at the same time in the tumour cells[70]. Alternatively, simultaneous targeted inhibition of both an essential protein component in the cancer cells and of endothelial cell-dependent tumour neovascularization aims not only at increased therapeutic potency, but also a reduction in the risk of molecular resistance formation by reducing the tumour cell population as a result of anti-angiogenic therapy[71]. Multi-targeted therapy can be achieved with either a combination of medicines or single 'promiscuous' drugs that act on a set of disease-relevant proteins[72]. Protein kinases, which share a relatively conserved ATP-binding site, are amenable to the latter concept of targeted poly-pharmacology.

The emerging shift towards multi-targeted kinase inhibitors can also be illustrated by a series of indolinone inhibitors developed for antitumour therapy in recent years (FIG. 6). The first of these compounds, SU5416, was originally developed as a monospecific inhibitor of vascular endothelial growth factor receptor (VEGFR) tyrosine kinase, which is involved in tumour angiogenesis[73]. The follow-up drug SU6668 had improved pharmacological properties and an increased potency as an anti-angiogenic agent, because it simultaneously inhibited three RTKs known to have a role in neovascularization: VEGFR2, PDGFRβ and FGFR1[74]. However, FGFR signalling was hardly affected by SU6668 in intact cells, and VEGFR2 seemed to be only partially blocked in a mouse model of angiogenesis, in contrast to the potent *in vivo* inhibition of the PDGFR by the kinase inhibitor[74,75]. SU6668 therefore did not attain balanced *in vivo* activity against its molecular targets, which is certainly a key aspect for effective multi-targeted therapy. Both SU5416 and SU6668 have not performed very well in clinical trials so far, which to some extent also relates to their respective pharmacokinetic profiles[76].

SU11248, the most recent drug out of this compound development line, shows comparable potency against several RTKs, including PDGFRα, PDGFRβ, VEGFR2, Kit and FLT3[77–79]. The balanced activity profile of this multi-targeted drug might well translate into simultaneous inhibition of disease-relevant RTKs *in vivo* and already seems to result in therapeutic efficacy in neoplasias such as renal cell carcinoma[71]. In addition, SU11248 showed promising results in GIST tumours with acquired resistance to imatinib[71]. Considering the

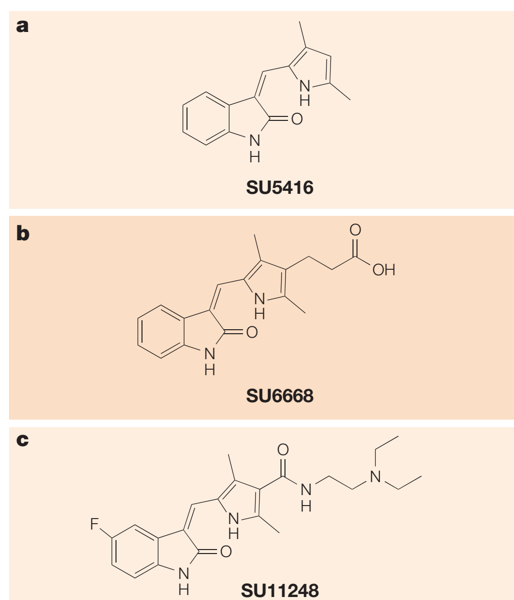

Figure 6 | **Chemical structures of the indolinone compounds.** SU5416 (**a**), SU6668 (**b**) and SU11248 (**c**). This series of ATP-competitive receptor tyrosine kinase (RTK) antagonists provides an example that illustrates a shift towards multi-targeted therapy with small-molecule kinase inhibitors. SU11248, the most recent compound out of this series, possesses balanced inhibitory activity against various RTKs, including platelet-derived growth factor receptor-α (PDGFRα), PDGFRβ, vascular endothelial growth factor receptor 2, Kit and FLT3[77–79].

structural aspects of both imatinib binding to its GIST target Kit and the interaction of SU11248-related indolinones with tyrosine kinases, SU11248 might be expected to override imatinib-desensitizing mutations in *KIT*, including the T670I substitution of the gatekeeper residue[37]. In addition to Kit in tumour cells, SU11248 seems to target both VEGFR and PDGFR tyrosine kinases in endothelial and perivascular cells, and interferes with tumour vascularization and, as a consequence, with the blood supply within the tumour[80]. The results of further clinical studies will clarify how efficacious multi-targeted kinase inhibitors such as SU11248 are in terms of potency and thwarting resistance formation.

There is, however, a drawback to this approach. The simultaneous inhibition of multiple targets with either 'promiscuous' small-molecule kinase inhibitors or cocktails of these drugs runs the risk of becoming too unselective; this might interfere with normal cellular function and result in dose-limiting toxicity. To keep potential adverse side effects to a minimum, compounds must ideally possess a multi-target selectivity that is restricted to cancer-relevant protein kinases and must be ineffective against proteins not linked to the actual disease. To address this challenge, a thorough analysis of the kinase selectivity of drug candidates must be undertaken. This can be done in high throughput by screening large collections of recombinant kinases against drug candidates in parallel[81,82].

Alternatively, this challenge might be addressed using proteomic techniques, which make use of immobilized kinase inhibitors for the affinity purification of cellular drug targets followed by sensitive mass spectrometry for subsequent protein identification[83–85]. Proteomic target-identification techniques that we have carried out often revealed that the targets of a drug belonged to different groups of the human protein kinase superfamily and were therefore fairly unrelated at the amino-acid level, as shown, for example, for a 'selective' receptor tyrosine kinase inhibitor, which also had cellular activity against several Ser/Thr kinases with roles in cell-cycle progression and anti-apoptotic signalling (H. Daub, unpublished results). There might therefore even be potential to develop potent multi-targeted drugs with rather 'counterintuitive' kinase inhibition profiles, which not only hit a group of closely related RTKs, but, for example, show balanced inhibitory activity against both tyrosine and Ser/Thr kinases involved in carcinogenesis.

Notably, the physiological outcome of multi-targeted therapies does not necessarily reflect the sum of the individual target contributions to cellular signalling[86]. For instance, simultaneous co-inhibition of the EGFR and HER2 RTKs with combinations of targeted agents results in regression of aneuploidy and restores a normal phenotype in ovarian carcinoma cells, which is not observed on ablation of either target alone[87]. Similar effects on the malignant phenotype of certain cancer cells might be achieved with single agents such as DUAL-SPECIFICITY KINASE INHIBITORS of these related RTKs, which are currently undergoing clinical evaluation[88].

Combinations or 'cocktails' of selective protein kinase inhibitors, with either other targeted agents or conventional chemotherapy, also represent an emerging therapeutic concept for preventing or overcoming resistance formation in human malignancies. These combinatorial approaches offer more flexibility with respect to target selection and therapeutic design than multi-targeted protein kinase inhibitors, because drugs with fundamentally different biological modes of action can be co-administered at different ratios relative to each other and according to variable time schedules. However, they require more effort to determine the optimal doses that are both efficacious and well tolerated by the treated patients. In the case of imatinib, combination with intensive traditional chemotherapy has yielded very promising results in Ph^+ ALL and the synergistic cytotoxic effect on the leukaemia cells seems to significantly reduce the risk of resistance formation inherent to imatinib monotherapy of this myeloproliferative disorder[89]. Furthermore, preclinical data support the idea that the combination of imatinib with targeted agents, such as farnesyltransferase inhibitors or heat-shock protein antagonists, can overcome resistance caused by imatinib-desensitizing mutations in the Bcr–Abl kinase domain[90,91]. There are, therefore, already several lines of evidence indicating that both new combination therapies, as well as multi-targeted protein kinase inhibitors, will become important elements in future cancer therapy and will have essential roles in preventing or overriding drug resistance in human malignancies.

DUAL-SPECIFICITY KINASE INHIBITOR
An inhibitor that specifically interferes with two distinct protein kinase activities.

Conclusions

The emergence of molecular resistance as a result of genetic alterations, which are frequently observed in *BCR–ABL*, seems to be a drawback of targeted treatment. It certainly poses a major challenge to successful anticancer therapy. However, resistance-conferring mutations can also be seen as the inevitable consequence of a drug-imposed selection process, which in fact confirms the validity of the targeted therapeutic approach. In the case of protein kinases, inhibitor-desensitizing mutations do not prevent the binding and catalytic utilization of ATP, and consequently permit the variant enzyme to compensate for the cellular function of non-resistant kinases in the presence of small-molecule antagonists. Therefore, mechanistic and structural insights into the molecular aspects of drug-target resistance provide a rationale for the selection and design of back-up compounds for drug development that show potent activity against mutant kinase alleles and might also be generally less susceptible to resistance formation.

It is essential to expand the definition of a disease-relevant target to include the whole range of functional mutant phenotypes. In this context, it might be conceivable that a set of distinct small-molecule inhibitors with complementary activities towards desensitized mutant alleles ensures effective inhibition of any resistant kinase variants that possibly emerge during targeted therapy. In this scenario, resistance formation against one targeted drug could always be therapeutically addressed with an alternative drug for the same cellular target. Moreover, the simultaneous inhibition of several cellular targets by poly-pharmacological intervention might have even greater potential in preventing the emergence of drug resistance in human malignancies. Poly-pharmacology with targeted agents in human cancer would be reminiscent of targeted combination therapies currently used for the treatment of viral diseases such as HIV infection to minimize the formation of drug resistance[92]. Hopefully, the future implementation of the discussed strategies to thwart resistance will result in more efficacious anticancer therapies and eventually shift the balance from disease relapse towards disease eradication.

1. Blume-Jensen, P. & Hunter, T. Oncogenic kinase signalling. *Nature* **411**, 355–365 (2001).
2. Kaelin, W. G. Jr. Gleevec: prototype or outlier? *Sci. STKE* **2004**, PE12 (2004).
3. Cohen, P. Protein kinases — the major drug targets of the twenty-first century? *Nature Rev. Drug Discov.* **1**, 309–315 (2002).
4. Dancey, J. & Sausville, E. A. Issues and progress with protein kinase inhibitors for cancer treatment. *Nature Rev. Drug Discov.* **2**, 296–313 (2003).
5. Gschwind, A., Fischer, O. M. & Ullrich, A. The discovery of receptor tyrosine kinases: targets for cancer therapy. *Nature Rev. Cancer* **4**, 361–370 (2004).
6. Coates, A., Hu, Y., Bax, R. & Page C. The future challenges facing the development of new antimicrobial drugs. *Nature Rev. Drug Discov.* **1**, 895–910 (2002).
7. Walsh, C. Molecular mechanisms that confer antibacterial drug resistance. *Nature* **406**, 775–781 (2000).
8. von Bubnoff, N., Peschel, C. & Duyster, J. Resistance of Philadelphia-chromosome positive leukemia towards the kinase inhibitor imatinib (STI571, Glivec): a targeted oncoprotein strikes back. *Leukemia* **17**, 829–838 (2003).
9. Cowan-Jacob, S. W. *et al.* Imatinib (STI571) resistance in chronic myelogenous leukemia: molecular basis of the underlying mechanisms and potential strategies for treatment. *Mini Rev. Med. Chem.* **4**, 285–299 (2004).
10. Hochhaus, A. & La Rosee, P. Imatinib therapy in chronic myelogenous leukemia: strategies to avoid and overcome resistance. *Leukemia* **18**, 1321–1331 (2004).
11. Nardi, V., Azam, M. & Daley, G. Q. Mechanisms and implications of imatinib resistance mutations in BCR–ABL. *Curr. Opin. Hematol.* **11**, 35–43 (2004).
12. Ross, D. M. & Hughes, T. P. Cancer treatment with kinase inhibitors: what have we learnt from imatinib? *Br. J. Cancer* **90**, 12–19 (2004).
13. Capdeville, R., Buchdunger, E., Zimmermann, J. & Matter, A. Glivec (STI571, imatinib), a rationally developed, targeted anticancer drug. *Nature Rev. Drug Discov.* **1**, 493–502 (2002).
14. Faderl, S. *et al.* The biology of chronic myeloid leukemia. *N. Engl. J. Med.* **341**, 164–172 (1999).
15. Sawyers, C. L. Chronic myeloid leukaemia. *N. Engl. J. Med.* **340**, 1330–1340 (1999).
16. Daley, G. Q., van Etten, R. A. & Baltimore, D. Induction of chronic myelogenous leukemia in mice by the $p210^{Bcr/Abl}$ gene of the Philadelphia chromosome. *Science* **247**, 824–830 (1990).
17. Lugo, T. G. *et al.* Tyrosine kinase activity and transformation potency of *Bcr–Abl* oncogene products. *Science* **247**, 1079–1082 (1990).
18. Druker, B. G. *et al.* Activity of a specific inhibitor of the BCR–ABL tyrosine kinase in the blast crisis of chronic myeloid leukemia and acute lymphoblastic leukemia with the Philadelphia chromosome. *N. Engl. J. Med.* **344**, 1038–1042 (2001).
19. Druker, B. J. *et al.* Effects of a selective inhibitor of the Abl tyrosine kinase on the growth of *Bcr–Abl* positive cells. *Nature Med.* **2**, 561–566 (1996).
20. Ottmann, O. G. *et al.* A phase 2 study of imatinib in patients with relapsed or refractory Philadelphia chromosome-positive acute lymphoid leukemias. *Blood* **100**, 1965–1971 (2002).
21. Druker, B. J. Imatinib as a paradigm of targeted therapies. *Adv. Cancer Res.* **91**, 1–30 (2004).
22. Hingorani, S. R. & Tuveson, D. A. Targeting oncogene dependence and resistance. *Cancer Cell* **3**, 414–417 (2003).
23. Gambacorti-Passerini, C. *et al.* α1 acid glycoprotein binds to imatinib (STI571) and substantially alters its pharmacokinetics in chronic myeloid leukemia patients. *Clin. Cancer Res.* **9**, 625–632 (2003).
24. Mahon, F. X. *et al.* *MDR1* gene overexpression confers resistance to imatinib mesylate in leukemia cell line models. *Blood* **101**, 2368–2373 (2003).
25. Thomas, J., Wang, L., Clark, R. E. & Pirmohamed, M. Active transport of imatinib into and out of cells: Implications for drug resistance. *Blood* 17 Aug 2004 (doi:10.1182/blood-2003-12-4276).
26. Donato, N. J. *et al.* BCR–ABL independence and LYN kinase overexpression in chronic myelogenous leukemia cells selected for resistance to STI571. *Blood* **101**, 690–698 (2003).
27. Dai, Y., Rahmani, M., Corey, S. J., Dent, P. & Grant, S. A Bcr/Abl-independent, Lyn-dependent form of imatinib mesylate (STI-571) resistance is associated with altered expression of Bcl-2. *J. Biol. Chem.* **279**, 34227–34239 (2004).
28. Gorre, M. E. *et al.* Clinical resistance to STI-571 cancer therapy caused by BCR–ABL gene mutation or amplification. *Science* **293**, 876–880 (2001).
29. Graham, S. M. *et al.* Primitive, quiescent, Philadelphia-positive stem cells from patients with chronic myeloid leukemia are insensitive to STI571 *in vitro*. *Blood* **99**, 319–325 (2002).
30. Branford, S. *et al.* High frequency of point mutations clustered within the adenosine triphosphate-binding region of BCR/ABL in patients with chronic myeloid leukemia or Ph-positive acute lymphoblastic leukemia who develop imatinib (STI571) resistance. *Blood* **99**, 3472–3475 (2002).
31. Hofmann, W. K. *et al.* Ph(+) acute lymphoblastic leukemia resistant to the tyrosine kinase inhibitor STI571 has a unique BCR–ABL gene mutation. *Blood* **99**, 1860–1862 (2002).
32. Roumiantsev, S. *et al.* 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. USA* **99**, 10700–10705 (2002).
33. Shah, N. P. *et al.* 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* **2**, 117–125 (2002).
An excellent paper about the amino-acid substitutions in Bcr–Abl from relapsed CML patients and the potential mechanisms how these mutations confer imatinib resistance.
34. von Bubnoff, N., Schneller, F., Peschel, C. & Duyster, J. BCR–ABL gene mutations in relation to clinical resistance of Philadelphia-chromosome-positive leukaemia to STI571: a prospective study. *Lancet* **359**, 487–491 (2002).
35. Corbin, A. S., La Rosee, P., Stoffregen, E. P., Druker, B. J. & Deininger, M. W. Several Bcr–Abl kinase domain mutants associated with imatinib mesylate resistance remain sensitive to imatinib. *Blood* **101**, 4611–4614 (2003).
36. Schindler, T. *et al.* Structural mechanism for STI-571 inhibition of abelson tyrosine kinase. *Science* **289**, 1938–1942 (2000).
First report of the co-crystal structure of an imatinib analogue in complex with the tyrosine kinase Abl.
37. Blencke, S. *et al.* Characterization of a conserved structural determinant controlling protein kinase sensitivity to selective inhibitors. *Chem. Biol.* **11**, 691–791 (2004).
This study provides an analysis of the 'gatekeeper' residue in several tyrosine kinases and shows the general relevance of this site for resistance formation against small-molecule inhibitors.
38. Nagar, B. *et al.* Crystal structures of the kinase domain of c-Abl in complex with the small molecule inhibitors PD173955 and imatinib (STI-571). *Cancer Res.* **62**, 4236–4243 (2002).
39. Branford, S. *et al.* Detection of BCR–ABL mutations in patients with CML treated with imatinib is virtually always accompanied by clinical resistance, and mutations in the ATP phosphate-binding loop (P-loop) are associated with a poor prognosis. *Blood* **102**, 276–832 (2003).
This study shows that the type of the imatinib resistance-inducing mutation in *BCR–ABL* predicts the clinical prognosis for relapsed CML patients.
40. Azam, M., Latek, R. R. & Daley, G. Q. Mechanisms of autoinhibition and STI-571/imatinib resistance revealed by mutagenesis of BCR–ABL. *Cell* **112**, 831–843 (2003).
This paper describes an interesting screening technique to identify potential mechanisms of resistance to targeted kinase inhibitors.
41. Apperley, J. F. *et al.* Response to imatinib mesylate in patients with chronic myeloproliferative diseases with rearrangements of the platelet-derived growth factor receptor beta. *N. Engl. J. Med.* **347**, 481–487 (2002).
42. Cools, J. *et al.* 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.* **348**, 1201–1214 (2003).
This study identifies a constitutively active PDGFRα variant as imatinib target in a haematologic disorder and further reports the emergence of imatinib resistance as a consequence of a mutation affecting the PDGFRα residue homologous to Thr315 in Abl.

43. Demetri, G. D. *et al.* Efficacy and safety of imatinib mesylate in advanced gastrointestinal stromal tumors. *N. Engl. J. Med.* **347**, 472–480 (2002).
44. Tamborini, E. *et al.* A new mutation in the KIT ATP pocket causes acquired resistance to imatinib in a gastrointestinal stromal tumor patient. *Gastroenterology* **127**, 294–299 (2004).
45. Wakai, T. *et al.* Late resistance to imatinib therapy in a metastatic gastrointestinal stromal tumour is associated with a second KIT mutation. *Br. J. Cancer* **90**, 2059–2061 (2004).
46. Ma, Y. *et al.* The c-KIT mutation causing human mastocytosis is resistant to STI571 and other KIT kinase inhibitors; kinases with enzymatic site mutations show different inhibitor sensitivity profiles than wild-type kinases and those with regulatory-type mutations. *Blood* **99**, 1741–1744 (2002).
47. Wisniewski, D. *et al.* Characterization of potent inhibitors of the Bcr–Abl and the c-kit receptor tyrosine kinases. *Cancer Res.* **62**, 4244–4255 (2002).
48. La Rosée, P., Corbin, A. S., Stoffregen, E. P., Deininger, M. W. & Druker, B. J. Activity of the Bcr–Abl kinase inhibitor PD180970 against clinically relevant Bcr–Abl isoforms that cause resistance to imatinib mesylate (Gleevec, STI571). *Cancer Res.* **62**, 7149–7153 (2002).
This is the first report demonstrating that many imatinib-restistant Bcr–Abl variants retain sensitivity to a structurally distinct kinase inhibitor.
49. Huron, D. R. *et al.* A novel pyridopyrimidine inhibitor of Abl kinase is a picomolar inhibitor of Bcr–Abl-driven K562 cells and is effective against STI571-resistant Bcr–Abl mutants. *Clin. Cancer Res.* **9**, 1267–1273 (2003).
50. von Bubnoff, N. *et al.* Inhibition of wild-type and mutant Bcr–Abl by pyrido-pyrimidine-type small molecule kinase inhibitors. *Cancer Res.* **63**, 6395–6404 (2003).
51. Kantarjian, H. M. *et al.* Dose escalation of imatinib mesylate can overcome resistance to standard-dose therapy in patients with chronic myelogenous leukemia. *Blood* **101**, 473–475 (2003).
52. Shah, N. P. *et al.* Overriding imatinib resistance with a novel ABL kinase inhibitor. *Science* **305**, 399–401 (2004).
53. O'Hare, T. *et al.* Inhibition of wild-type and mutant Bcr–Abl by AP23464, a potent ATP-based oncogenic protein kinase inhibitor: Implications for CML. *Blood* **104**, 2532–2539 (2004).
67. Roche-Lestienne, C. *et al.* Several types of mutations of the Abl gene can be found in chronic myeloid leukemia patients resistant to STI571, and they can pre-exist to the onset of treatment. *Blood* **100**, 1014–1018 (2002).
68. Roche-Lestienne, C., Lai, J. L., Darre, S., Facon T. & Preudhomme, C. A mutation conferring resistance to imatinib at the time of diagnosis of chronic myelogenous leukemia. *N. Engl. J. Med.* **348**, 2265–2266 (2003).
69. Hofmann, W. K. *et al.* Presence of the BCR–ABL mutation Glu255Lys prior to STI571 (imatinib) treatment in patients with Ph+ acute lymphoblastic leukemia. *Blood* **102**, 659–661 (2003).
54. Manning, G., Whyte, D. B., Martinez, R., Hunter, T. & Sudarsanam, S. The protein kinase complement of the human genome. *Science* **298**, 1912–1934 (2002).
55. Eyers, P. A., Craxton, M., Morrice, N., Cohen, P. & Goedert, M. Conversion of SB 203580-insensitive MAP kinase family members to drug-sensitive forms by a single amino-acid substitution. *Chem. Biol.* **5**, 321–328 (1998).
56. Liu, Y. *et al.* Structural basis for selective inhibition of Src family kinases by PP1. *Chem. Biol.* **8**, 257–266 (1999).
57. Blencke, S., Ullrich, A. & Daub, H. Mutation of threonine 766 in the epidermal growth factor receptor reveals a hotspot for resistance formation against selective tyrosine kinase inhibitors. *J. Biol. Chem.* **278**, 15435–15440 (2003).
58. 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.* **277**, 46265–46272 (2002).
59. Muhsin, M., Graham, J. & Kirkpatrick, P. Gefitinib. *Nature Rev. Drug Discov.* **2**, 515–516 (2003).
60. Cohen, M. H. *et al.* United States Food and Drug Administration Drug Approval summary: Gefitinib (ZD1839; Iressa) tablets. *Clin. Cancer Res.* **10**, 1212–1218 (2004).
61. Lynch, T. J. *et al.* Activating mutations in the epidermal growth factor receptor underlying responsiveness of non-small-cell lung cancer to gefitinib. *N. Engl. J. Med.* **350**, 2129–2139 (2004).
This paper and reference 62 were the first to describe a correlation of drug-sensitizing, activating mutations in the *EGFR* gene with clinical responses to gefitinib in lung cancer patients.
62. Paez, J. G. *et al.* EGFR mutations in lung cancer: correlation with clinical response to gefitinib therapy. *Science* **304**, 1497–1500 (2004).
63. Pao, W. *et al.* 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. USA* **101**, 13306–13311 (2004).
64. Sordella, R., Bell, D. W., Haber, D. A. & Settleman, J. Gefitinib-sensitizing EGFR mutations in lung cancer activate anti-apoptotic pathways. *Science* **305**, 1163–1167 (2004).
65. Cools, J. *et al.* PKC412 overcomes resistance to imatinib in a murine model of FIP1L1-PDGFRα-induced myeloproliferative disease. *Cancer Cell* **3**, 459–469 (2003).
66. Mohammadi, M. *et al.* Structure of the tyrosine kinase domain of fibroblast growth factor receptor in complex with inhibitors. *Science* **276**, 955–960 (1997).
70. Druker, B. J. Overcoming resistance to imatinib by combining targeted agents. *Mol. Cancer Ther.* **2**, 225–226 (2003).
71. Hampton, T. 'Promiscuous' anticancer drugs that hit multiple targets may thwart resistance. *JAMA* **292**, 419–22 (2004).
72. Morphy, R., Kay, C. & Rankovic, Z. From magic bullets to designed multiple ligands. *Drug Discov. Today* **9**, 641–651 (2004).
73. Fong, T. A. *et al.* SU5416 is a potent and selective inhibitor of the vascular endothelial growth factor receptor (Flk-1/KDR) that inhibits tyrosine kinase catalysis, tumor vascularization, and growth of multiple tumor types. *Cancer Res.* **59**, 99–106 (1999).
74. Laird, A. D. *et al.* SU6668 is a potent antiangiogenic and antitumor agent that induces regression of established tumors. *Cancer Res.* **60**, 4152–4162 (2000).
75. Bergers, G., Song, S., Meyer-Morse, N., Bergsland, E. & Hanahan, D. Benefits of targeting both pericytes and endothelial cells in the tumor vasculature with kinase inhibitors. *J. Clin. Invest.* **111**, 1287–1295 (2003).
76. Eskens, F. A. Angiogenesis inhibitors in clinical development; where are we now and where are we going? *Br. J. Cancer* **90**, 1–7 (2004).
77. Abrams, T. J., Lee, L. B., Murray, L. J., Pryer, N. K. & Cherrington, J. M. SU11248 inhibits KIT and platelet-derived growth factor receptor beta in preclinical models of human small cell lung cancer. *Mol. Cancer Ther.* **2**, 471–478 (2003).
78. Mendel, D. B. *et al. In vivo* antitumor activity of SU11248, a novel tyrosine kinase inhibitor targeting vascular endothelial growth factor and platelet-derived growth factor receptors: determination of a pharmacokinetic/pharmacodynamic relationship. *Clin. Cancer Res.* **9**, 327–337 (2003).
79. O'Farrell, A. M. *et al.* SU11248 is a novel FLT3 tyrosine kinase inhibitor with potent activity *in vitro* and *in vivo*. *Blood* **101**, 3597–3605 (2003).
80. Schueneman, A. J. *et al.* SU11248 maintenance therapy prevents tumor regrowth after fractionated irradiation of murine tumor models. *Cancer Res.* **63**, 4009–4016 (2003).
81. Davies, S. P., Reddy, H., Caivano, M. & Cohen, P. Specificity and mechanism of action of some commonly used protein kinase inhibitors. *Biochem. J.* **351**, 95–105 (2000).
82. Bain, J., McLauchlan, H., Elliott, M. & Cohen, P. The specificities of protein kinase inhibitors: an update. *Biochem. J.* **371**, 199–204 (2003).
83. Godl, K. *et al.* An efficient proteomics method to identify the cellular targets of protein kinase inhibitors. *Proc. Natl Acad. Sci. USA* **100**, 15434–15439 (2003).
84. Brehmer, D., Godl, K., Zech, B., Wissing, J. & Daub, H. Proteome-wide identification of cellular targets affected by bisindolylmaleimide-type protein kinase C inhibitors. *Mol. Cell. Proteomics* **3**, 490–500 (2004).
85. Daub, H., Godl, K., Brehmer, D., Klebl, B. & Müller, G. Evaluation of kinase inhibitor selectivity by chemical proteomics. *Assay Drug. Dev. Technol.* **2**, 215–224 (2004).
86. Cheok, M. H. *et al.* Treatment-specific changes in gene expression discriminate *in vivo* drug response in human leukemia cells. *Nature Genet.* **34**, 85–90 (2003).
87. Pack, S. D. *et al.* Simultaneous suppression of epidermal growth factor receptor and c-erbB-2 reverses aneuploidy and malignant phenotype of a human ovarian carcinoma cell line. *Cancer Res.* **64**, 789–794 (2004).
This interesting paper reports the reversal of aneuploidy after co-targeting of the EGFR and the closely related HER2 tyrosine kinase.
88. Baselga, J. & Hammond, L. A. HER-targeted tyrosine-kinase inhibitors. *Oncology* **63** (Suppl. 1), 6–16 (2002).
89. Towatari, M. *et al.* Combination of intensive chemotherapy and imatinib can rapidly induce high-quality complete remission for a majority of patients with newly diagnosed BCR–ABL positive acute lymphoblastic leukemia. *Blood* 17 Aug 2004 (doi:182/blood-2004-04-1389)
90. Hoover, R. R., Mahon, F. X., Melo, J. V. & Daley, G. Q. Overcoming STI571 resistance with the farnesyl transferase inhibitor SCH66336. *Blood* **100**, 1068–1071 (2002).
91. Gorre, M. E., Ellwood-Yen, K., Chiosis, G., Rosen, N. & Sawyers, C. L. BCR–ABL point mutants isolated from patients with imatinib mesylate-resistant chronic myeloid leukemia remain sensitive to inhibitors of the BCR–ABL chaperone heat shock protein 90. *Blood* **100**, 3041–3044 (2002).
52. Richman, D. D. HIV chemotherapy. *Nature* **410**, 995–1001 (2001).

Acknowledgements

The authors wish to thank D. Brehmer for stimulating discussions and his contributions to the illustrations in figure 2 and figure 5. The work carried out in the laboratory of H.D. is supported by a grant from the German Ministry for Education and Research (Bundesministerium für Bildung und Forschung, BMBF).

Competing interests statement

The authors declare competing financial interests: see Web version for details.

Online links

DATABASES

The following terms in this article are linked online to:

Entrez Gene:
http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=gene
ABL | *BCR* | EGFR | FGFR1 | FIP1L1 | FLT3 | insulin-like growth factor 1 receptor | Kit | Lyn | PDGFRα | PDGFRβ | P-glycoprotein | VEGFR

OMIM: http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=OMIM
CML

Cancer.gov: http://www.cancer.gov/cancer_information/
Adult ALL

Access to this interactive links box is free online.