Acta Pharmaceutica Sinica B 2011;1(4):197–207

Institute of Materia Medica, Chinese Academy of Medical Sciences
Chinese Pharmaceutical Association

Acta Pharmaceutica Sinica B

www.elsevier.com/locate/apsb
www.sciencedirect.com

REVIEW

Mechanisms of acquired resistance to tyrosine kinase inhibitors

Yi-fan Chen, Li-wu Fu*

State Key Laboratory of Oncology in Southern China, Cancer Center, Sun Yat-sen University, Guangzhou 510060, China

Received 23 September 2011; revised 11 October 2011; accepted 17 October 2011

KEY WORDS

Tyrosine kinase;
Tyrosine kinase inhibitor;
TKI-resistance mechanism

Abstract In recent years, structural and functional studies reveal that tyrosine kinases (TKs) act as the essential components of signal transduction pathways that regulate cancer cell proliferation, apoptosis and angiogenesis, and therefore become potential targets for anticancer therapy. Most of TK inhibitors (TKIs) are small molecular and hydrophobic compounds, thus they can rapidly reach their specific intracellular targets and inhibit the activation of the related TKs. Unfortunately, accompanied with patients who gain great benefit of TKIs therapy, increasing evidences of acquired resistance to these agents have been documented. The unveiling point mutations within the kinase domain, gene amplification or overexpression, or modification of signaling pathway have been implicated in drug resistance. Additionally, overexpression of ABC transporters is likely to set stage for resistant development. In this review, we focus on the discussion of the molecular mechanisms of acquired resistance to TKIs therapy. The mechanistic understanding may help to put forward new hypotheses on drug development and design better therapies to overcome TKIs resistance.

Abbreviations: TK, tyrosine kinase; TKI, tyrosine kinase inhibitor; ABC transporter, ATP-binding cassette transporter; FDA, food and drug administration; RTK, receptor tyrosine kinase; NRTK, non- receptor tyrosine kinase; EGFR, epidermal growth factor receptor; FLT3, Fms-like tyrosine kinase 3; PDGFR, platelet-derived growth factor receptor; VEGFR, vascular endothelial growth factor receptor; CML, chronic myelogenous leukemia; BCR–ABL, a fusion gene is created by juxtapositioning the *Abl1* gene on chromosome 9 to a part of the *BCR* ("breakpoint cluster region") gene on chromosome 22; GISTs, gastrointestinal stromal tumors; Ph^+ALL, Philadelphia chromosome-positive acute lymphoblastic leukemia; NSCLC, non-small-cell lung cancer; PDGFR, platelet-derived growth factor receptor; MRP1, multidrug resistance protein 1; ABCG2, breast cancer resistance protein; hOCT1, human organic cation transporter 1

*Corresponding author. Tel.: +86 20 87343163; fax: +86 20 87343170.
E-mail address: Fulw@mail.sysu.edu.cn (Li-wu Fu).

Peer review under responsibility of Institute of Materia Medica, Chinese Academy of Medical Sciences and Chinese Pharmaceutical Association.
doi:10.1016/j.apsb.2011.10.007

1. Introduction

Carcinogenesis in numerous cases is based on a pathological intracellular signal transduction, in which the activation of specific tyrosine kinases plays a major role including regulation of cell growth, differentiation, adhesion, motility, death and so on[1,2]. Mutations in TKs and aberrant activation of their intracellular signaling pathways have been causally linked to cancers. So the connection has driven the development of a new generation of drugs that block or attenuate TKs activity, providing a broader therapeutic window with less toxicity and high efficiency. Nowadays, targeted therapies represent an integrative approach to cancer therapy that has already led to important clinical results. Although several TKIs have been approved by Food and Drug Administration (FDA) and applied in the clinic or in the clinical trial, increasing evidences have shown that cancer cells treated with TKIs tend to acquire genetic modifications to escape the inhibition from these agents. Insight into the molecular events underlying TKI-resistance is needed for the development of new treatment approaches, such as next generation TKIs, despite the mechanisms are varied and some of them are uncertain. In this review, we summarize the molecular mechanisms of acquired resistance to TKIs therapy.

2. Tyrosine kinases

The human protein kinase genome (also known as the kinome) contains 518 protein kinase genes, including genes that encode transmembrane receptor tyrosine kinases (RTKs) and soluble cytoplasmic tyrosine kinases (also known as non-receptor tyrosine kinases, NRTKs)[3,4]. Humans have 58 known RTKs which fall into 20 subfamilies, including the well-known insulin receptor, Fms-like tyrosine kinase 3 (FLT3), epidermal growth factor receptor (EGFR), platelet-derived growth factor receptor (PDGFR)[3,5]. All RTKs have a similar molecular architecture, with ligand-binding domains in the extracellular region, a single transmembrane helix, and a cytoplasmic region that contains the protein tyrosine kinase (TK) domain plus additional carboxy (C-) terminal and juxtamembrane regulatory regions[6]. Activation of RTKs is initiated by hormones or binding of growth factors to specific sites within the extracellular domain of the receptor. Upon ligand binding, RTKs undergo a dimerization process (or a conformational change), i.e., a bivalent ligand interacts simultaneously with two receptor molecules and effectively crosslinks them into a dimeric complex[3,6], resulting in autophosphorylation of the tyrosine kinase domains[3,7]. Most tyrosine autophosphorylation sites are located in non-catalytic regions of the receptor molecule and function as binding sites for Src homology 2 (SH2) or phosphotyrosine binding (PTB) domains of a variety of signaling proteins[7]. Then the phosphotyrosine residues in the cytoplasmic regions of RTKs are recognized as docking sites by signaling factors such as PLCγ1 through their SH2 domains and hence link PTK activation to downstream signaling pathways[8].

On the other hand, NRTKs account for third of the approximately 90 known TKs which fall into 10 subfamilies based on kinase domain sequence, including the well-characterized Src, c-Abl, JAK. They are lack of transmembrane domains and are found in the cytosol, the nucleus, and the inner surface of the plasma membrane[9]. Normally, NRTKs maintained in an inactive state through multiple mechanisms, including binding of inhibitory proteins or lipids or intramolecular autoinhibition[10]. Activation of NRKTs occurs through binding to transmembrane receptors or a variety of intracellular signals including dissociation of inhibitors or transphosphorylation by other tyrosine kinases. Upon tyrosine phosphorylaton activation, immunoreceptor tyrosine-based activation motifs (ITAMs) serve as a docking site for downstream signaling molecules and adapter proteins containing SH2 or phosphotyrosine binding domains, leading to multiple cascades of signal transmission[11]. The involvement of NRTKs in cancer can occur through various mechanisms such as overexpression, mutation, and translocation; and therefore, many compounds have been developed attempting to inhibit their activity[12].

Taken together, the TKs' functions are as a point of convergence for diverse signaling pathways and define key biological outcomes, such as cell proliferation, differentiation, motility and survival, in response to a wide range of physiological stimuli[8]. Phosphorylation of tyrosine residues in target TKs is essential for maintaining cellular homeostasis and modulating gene expression in various intercellular and intracellular signaling pathways. TKs are therefore important targets for basic research and drug development[3,5].

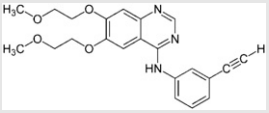
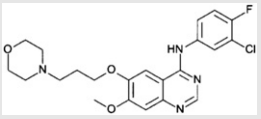
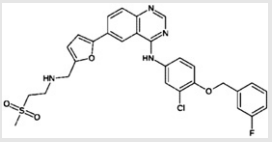
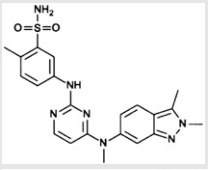
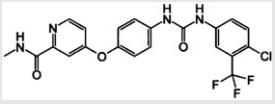
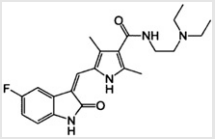
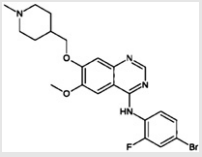
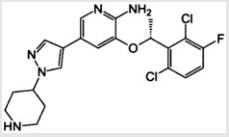
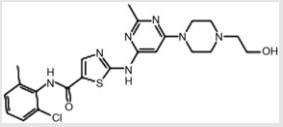
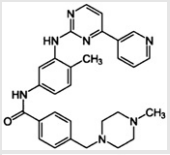
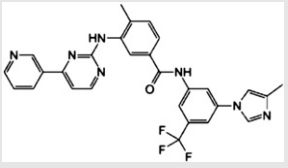
3. Small molecule tyrosine kinase inhibitors

Alterations of TKs signal transduction found in proliferative disorders lead to the hypothesis that tyrosine kinase inhibitors (TKIs) could have anticancer effects and, as a result, the development of TKIs has become a hot area of anticancer drug research.

The idea behind much of anti-TK drugs discovery is to find small molecules that directly inhibit the catalytic activity of the kinase by interfering with the binding of ATP or substrates[9]. Generally, most of these TKIs can be categorized into four groups: (1) ATP-competitive inhibitors, which bind predominantly to the ATP-binding site of the kinase when this site is in the active conformation; (2) inhibitors that recognize and bind to the non-active conformation of the ATP-binding site of the kinase, thus making activation energetically unfavorable; (3) allosteric inhibitors, that bind outside of the ATP-binding site, modifying the tridimensional structure of the receptor and disrupting the interaction between the ATP and the kinase pocket; and (4) covalent inhibitors, that bind irreversibly by covalently bonding to the ATP-binding site of the target kinase[12]. Some TKIs target a wide range of kinase, such as Imatinib, which is a drug used to treat certain types of cancer. By 2011, Imatinib has been approved by FDA to treat ten different cancers, including all stages of CML, GISTs and Ph^+ B-ALL. It is unclear whether this lack of selectivity should be considered an advantage or a shortcoming versus non-selective TKIs. However, many receptor tyrosine kinase pathways (for example, PDGF, EGF and VEGF) are simultaneously activated, suggesting that multi-target TKIs could be superior to selective inhibitors of a single receptor[3].

Until 2011, 11 tyrosine kinase inhibitors have received US Food and Drug Administration approval as cancer treatments. There are considerable efforts to develop small molecule inhibitors for a host of other kinases that are implicated in cancer and other diseases. Here we have summarized the information of the TKIs in Table 1[13].

Table 1 Tyrosine kinase inhibitor approved by FDA until 2011.

Name	Alternative name	Targets	Clinical application	Chemical structure
Receptor tyrosine kinase inhibitors				
Erlotinib	Tarceva, OSI-774	EGFR	Advanced or metastatic NSCLC, pancreatic cancer	
Gefitinib	Iressa, ZD1839	EGFR	Advanced or metastatic NSCLC	
Lapatinib	Tykerb, GW 572016	EGFR, HER2	HER-2$^+$ advanced or metastatic breast cancer	
Pazopanib	Votrient, GW-786034	VEGFRs, PDGFRs, c-Kit	Advanced RCC	
Sorafenib	Nexavar, BAY43-9006	Raf kinase, VEGFR2, c-Kit, FLT3, PDGFR-β	Advanced RCC, advanced HCC	
Sunitinib	Sutent SU11248	VEGFR, PDGFR, c-Kit, FLT3, CSF-1R	Imatinib–resistant or intolerant GISTs, metastatic RCC	
Vandetanib	Caprelsa ZD6474	VEGFR, EGFR	Metastatic medullary thyroid cancer	
Non-receptor tyrosine kinase inhibitors				
Crizotinib	Xalkori	ALK	Advanced or metastatic ALK$^+$ NSCLC	
Dasatinib	Sprycel, BMS 354825	BCR–ABL, SRC family, c-Kit, EPHA2, PDGFR	All phases of CML with resistance to prior therapy including imatinib, Ph$^+$ALL with resistance to prior therapy	
Imatinib	Gleevec, STI571	BCR–ABL, c-Kit, PDGFR	All stages of CML, GISTs, Ph$^+$ B-ALL, dermatofibrosarcoma	
Nilotinib	Tasigna, AMN107	BCR–ABL, PDGFR, c-Kit	Chronic phase and accelerated phase Ph$^+$ CML in adult patients resistant to prior therapy, GISTs	

Adapted from Wang et al.[13].

Although chemists continue to develop novel cytotoxic agents with unique mechanisms of action, many of these compounds are still limited by their general toxicity to proliferating cells, including some normal cells[14]. Targeted therapy provides a new approach for cancer therapy that has the potential for avoiding some of the drawbacks associated with cytotoxic chemotherapy[15]. While most novel, target directed cancer drugs have pregenomic origins, one can anticipate a post genomic wave of sophisticated "smart drugs" to fundamentally change the treatment of all cancers[16]. However, clinical and *in vitro* evidences have shown that cells treated with TKIs tend to acquire genetic modifications resulting in resistance to these agents. Better understanding of the mechanism of action of a drug may also help in defining potential mechanisms of resistance. Clearly, the lessons learnt from the targeted agents can aid the design and evaluation of next generation compounds[17].

4. Mechanisms of resistance to TKIs

4.1. Mutations

Up to now, more than 100 mutations have been described affecting more than 70 amino acids causing resistance by heterogeneous molecular mechanisms[18]. The most common and prevalent mechanism leading to against TKIs therapy is point mutations within the kinase domain, which decrease the affinity of the TKIs to binding domain. Some mutations may occur around the binding site, which make extensive conformational changes, thereby impeding TKIs approach through steric hindrance. Moreover, some mutations may render the predominance of ATP to competitive binding to the kinase compare with the second generation TKIs, such as Dasatinib, Nilotinib or Bosutinib[19].

4.1.1. T790M mutations induced by Gefitinib

The strongest evidences come from Gefitinib, which mimics adenosine triphosphate (ATP) and is found to bind with high affinity to EGFR kinase in a competitive manner[20]. About 70–80% of non-small cell lung cancers harbor a somatic mutation in the tyrosine kinase domain of the EGFR gene that responds to Gefitinib. EGFR mutation usually occurs in the first 4 exons of the tyrosine kinase domain, and a deletion involving 5 amino acids (codons 746–750) together with a point mutation at codon 858 (L858R: replaces leucine 858 with arginine) account for 90% of all EGFR mutations[20,21]. This somatic mutation, which seems to arise more frequently in women and in Asians, is correlated with dramatic clinical responses to treatment with Gefitinib[22]. It competitively inhibits the binding of ATP to the EGFR kinase, resulting in inhibition of phosphorylation, disrupting downstream signaling and inducing cell apoptosis. Strikingly, direct binding measurements show that Gefitinib binds 20-fold more tightly to the L858R mutant than to the wild-type enzyme and produce an initially dramatic response in lung cancer patients harboring the L858R mutant[20,23].

However, patients with EGFR-mutant lung adenocarcinoma develop acquired resistance to Gefitinib after a median of 10–16 months. More than 60% of these cases harbor a second EGFR mutation, T790M (threonine-to-methionine mutation at codon 790 in EGFR), who have the Gefitinib refractory, additionally; other secondary resistance mutations (D761Y, L747S, T854A) seem to be rare[24–28]. The T790 in EGFR is located at a key position in the ATP binding cleft, often referred to as the "gatekeeper residue." And the molecular mechanism of TKI-resistance is that the ATP affinity of the oncogenic L858R mutant is increased by more than an order of magnitude, leading to resistance to Gefitinib. In a human bronchial epithelial cell line, overexpression of EGFR T790M confers a growth advantage over cells expressing wild type EGFR[29]. The development of acquired resistance by the T790M substitution may be modeled in two ways. In one model, the T790M substitution is absent in the initial tumor cell population and rises *de novo* in one or more clonal populations upon treatment with an EGFR TKI. In the second model, the T790M substitution pre-exists *in cis* with a primary activating mutation in a small population and is subjected to positive selection pressure in the presence of an EGFR TKI[30].

The T790M mutation also possesses enhanced phosphorylating activity, especially in combination with the L858R mutation, leading lung cancer cell to survival which indicates that the T790M mutant is actually an oncogene[20]. Regales et al.[29] developed mice with inducible expression in type II pneumocytes of EGFR T790M alone or together with a drug-sensitive L858R mutation. Both transgenic lines develop lung adenocarcinomas that require mutant EGFR for tumor maintenance but are resistant to an EGFR kinase inhibitor. Notably, EGFR T790M-expressing animals develop tumors with longer latency than EGFR L858R+T790M-bearing mice and in the absence of additional kinase domain mutations[29]. Interestingly, EGFR T790M alleles have been detected at lower frequency in untreated NSCLCs, suggesting that they may confer oncogenic activity to EGFR in addition to their role in acquired drug resistance[31]. Additionally, germ line T790M mutation has been detected in a family that exhibits inherited predisposition to lung adenocarcinoma[32]. These properties may explain that it is initial presence before drug selection and its rapid selection as the single drug resistance mutation during therapy with Gefitinib[22]. So a deeper understanding of the molecular and cellular basis of this phenomenon is crucial to the future development of alternative therapies to overcome this resistance[33].

4.1.2. T315I mutation induced by Imatinib

Point mutation within the BCR–ABL kinase domain is another major cause of acquired resistance. Licensed tyrosine kinase inhibitors are ineffective against these mutations and their development reduces life expectancy of CML in chronic phase from 10 years to just 22 months[34]. Around 30% of patients with CML will have to stop Imatinib therapy due to intolerance and resistance[35,36]. The *in vitro* data suggest that Imatinib treatment confers the mutant cell clone with increased oncogenic fitness.

T315I mutation (resulting in substitution of Ile for a Thr residue at the "gatekeeper" position 315) is at a higher frequency than other amino-acid substitutions and is responsible for 14% of reported cases occur at certain sites[37]. Furthermore, T315I mutation raises particular concern, because it also provides resistance to second-generation kinase inhibitors already approved for clinical use (Nilotinib and Dasatinib; see Tables 1 and 2)[36]. Threonine at position 315 forms a crucial hydrogen bond with Imatinib and the absence of an oxygen atom in the substituted isoleucine prevented

Table 2 *In vitro* sensitivity of non-mutant and mutant BCR–ABL against TKIs.

Name	Imatinib (nmol/L)	Nilotinib (nmol/L)	Dasatinib (nmol/L)
Native BCR–ABL	*260*	*13*	*0.8*
M244V	**2000**	*38*	*1.3*
G250E	**1350**	*48*	*1.8*
Q252H	**1325**	**70**	**3.4**
Y253F	***3475***	**125**	*1.4*
Y253H	***>6400***	**450**	*1.3*
E255K	***5200***	**200**	**5.6**
E255V	***>6400***	**430**	**11**
V299L	*540*	N/A	**18**
F311L	*480*	*23*	*1.3*
T315A	*971*	**61**	***125***
T315I	***>6400***	***>2000***	***>200***
F317L	**1050**	*50*	**7.4**
F317V	*350*	N/A	**53**
M351T	*880*	*15*	*1.1*
E355G	**2300**	N/A	*1.8*
F359V	**1825**	**175**	*2.2*
V379I	**1630**	**51**	*0.8*
L387M	*1000*	*49*	*2*
H396P	*350*	*41*	*0.6*
H396R	**1750**	*41*	*1.3*

Quoted from La Rosée et al.[36].
Note: Imatinib-sensitive, ≤1000 (nmol/L) (italic); intermediate-sensitive ≤3000 (nmol/L) (bold); insensitive >3000 (nmol/L) (bold italic). Nilotinib-sensitive ≤50 (nmol/L); intermediate sensitive ≤500 (nmol/L); insensitive >500 (nmol/L). Dasatinib-sensitive ≤3 (nmol/L); intermediate sensitive ≤60 (nmol/L); insensitive >60 (nmol/L).
Abbreviation: N/A, no data available.

bond formation[37]. Additionally, X-ray crystallography has revealed how single point mutations in the various domains of the kinase pocket can affect Imatinib binding[36]. T315I mutation confers resistance by blocking Imatinib access through steric hindrance and/or removing of critical hydrogen bonds, which is resistant to all currently approved BCR–ABL kinase inhibitors. Various other strategies are in use to optimize the treatment of CML, including dose optimization of Imatinib, combination therapy, and use of maintenance therapy with interferon-alpha and vaccines[38,39].

4.1.3. *Other mutations induced by related TKIs*

Therapeutic inhibition of KIT/PDGFRA kinase activity by Imatinib has emerged as the first-line treatment option in patients with inoperable gastrointestinal stromal tumor (GIST)[40]. However, Imatinib response depends on KIT/PDGFRA mutational status. Most primary mutations of KIT and PDGFRA in GIST are sensitive to Imatinib and resistance occurs in most cases because of the acquisition (or emergence through secondary to the selective pressure) of secondary mutations[41]. Secondary mutations in KIT exon 14 (kinase domain), exon 17 (activation loop: D816V/H, D820Y, N822Y/K, Y823D) and in PDGFRA exon 14 (D842V, ATP binding site: T670I) confer Imatinib and Sunitinib-resistance occur in GIST[42–48]. Like Imatinib, Sunitinib targets the inactivated conformation of KIT, PDGFRA kinases and binds with high affinity to the ATP binding pocket. Both of them could be effectively against the activation of the primary mutations of KIT and PDGFRA. However, unlike Imatinib, Sunitinib does not access the deep hydrophobic part of the ATP binding site, which explains some differences in the inhibitory properties of Sunitinib, like the potency against secondary mutations[49]. After emergence secondary mutations in KIT and PDGFRA, the kinases show a reduced binding affinity to the Imatinib or Sunitinib and still retain the activity

Similarly, activating mutations in the FLT3 are one of the most common molecular abnormalities found in *de novo* acute myeloid leukemia (AML) and have a strong negative prognostic impact[50]. FLT3 mutations occur within two specific regions of the FLT3 gene (juxtamembrane (JM) domain and tyrosine kinase domain)[51]. The most common type of FLT3 mutations is that of internal tandem duplication (FLT3/ITD) in the JM domain, which occurs in up to 30% of patients with AML and in 5% of patients with myelodysplastic syndrome, whereas point mutations in the TK domain are observed in approximately 7% of patients with AML[52]. Both mutations represent gain-of-function alterations after treated with TKIs, which render the kinase less accessible to the inhibitors, leading to the constitutive activation of FLT3 and the potent proliferation of leukemic cells.

Some of the best-studied FLT3 targeted inhibitors to date include PKC412, SU5614, Sorafenib and Sunitinib[53]. Despite their remarkable efficacy in reducing the leukemic clone in a subset of patients with AML, remission in patients who have had single-agent therapy tends to be short and secondary resistance develops rapidly[54]. A screening assay (see Table 3) used to study resistance profiles of three FLT3 inhibitors, PKC412, SU5614 and Sorafenib, showed non-overlapping mechanisms of resistance for these inhibitors. In contrast, an overlapping resistance profiles displayed for ABL inhibitors, namely Imatinib, Nilotinib and Dasatinib, show incredible high resistance to the T315I gatekeeper mutation (see Table 2)[55]. As a result, a combination of FLT3 inhibitors might be beneficial to the patients who acquired FLT3 resistance mutations[56–59].

Further studies reveal that a great majority of somatic mutations in PIK3Cα (PIK3CA) are missense mutations clustering in exons 9 and 20 in patients with NSCLC by EGFR-TKIs treated. These mutant exons encode a part of the helical and kinase domains, respectively. E545K and M1043I point mutation are detected in the heterozygous mutation exons. Mutant PIK3Cα stimulates the PI3K/AKT1 pathway and promotes cell growth in several cancers. [^{3}H]-thymidine incorporation data suggests that PIKC3α, but not PIKC3β or PIKC3γ plays a role in the Imatinib-resistance, resulting in constitutive activation and oncogenicity[60,61]. In addition, novel rarely mutations are detected after treated with new developed TKIs. The fusion gene EML4-ALK (echinoderm microtubule-associated protein-like 4 gene and the anaplastic lymphoma kinase gene) is recently identified as a novel genetic alteration in NSCLC, which has a strong oncogenic activity both *in vitro* and *in vivo* and may be associated with resistance to Erlotinib treatment[62]. Furthermore, from a Gefitinib-resistant patient carrying the activating L858R mutation, Costa et al.[63] identified a novel secondary resistant mutation, L747S *in cis* to the activating mutation, which attenuated the up-regulation of Bim (Bcl-2 interacting mediator of cell death) and reduced apoptosis.

Why do patients acquire these mutations during or after TKIs therapy? Although the mechanisms are not very clear, one explanation for these phenomena is that specific TKIs

Table 3 *In vitro* sensitivity of native FLT3 and mutant FLT3-ITD or -TKD against TKIs.

Name	PKC412 (nmol/L)	SU5614 (nmol/L)	Sorafinib (nmol/L)
Native FLT3	*8*	*100*	*8*
A627T	***97***	N/A	N/A
N676D	***235***	**400**	*100*
N676I	***40***	**400**	*40*
N676K	***100***	N/A	N/A
N676S	***25***	*100*	*10*
F691I	***121***	***>2000***	***>1000***
F691L	*10*	**300**	***>1000***
G697R	***>400***	N/A	N/A
G697S	***53***	N/A	N/A
C825S	**17.5**	**300**	*9*
D835E	*10*	**350**	*49*
D835N	N/A	***1000***	N/A
D835Y	**15**	***500***	*49*
D839G	**20**	**350**	*10*
D839H	*10*	**300**	*80*
S451F	***48***	N/A	N/A
S84IC	*8*	*125*	*10*
Y842C	*4*	***500***	*10*
Y842D	*2*	**300**	**250**
Y842H	*4*	***700***	**300**
Y842N	*9*	***1000***	***600***
Y842S	*4*	***1000***	**400**
M855T	**20**	**325**	*10*

Adapted from von Bubnoff et al.[53,54,56–59].
Note: PKC412-sensitive <12.5 (nmol/L) (italic); intermediate-sensitive <25.0 (nmol/L) (bold); insensitive ≥25 (nmol/L) (bold italic). SU5614, Sorafenib-sensitive <250 (nmol/L); intermediate sensitive <500 (nmol/L); insensitive ≥500 (nmol/L).
Abbreviation: N/A, no data available.

treatment help tumor to select the preexisting cell population, which has a selective advantage[24]. In addition, the advent inhibitors increase patients' genetic instability that promotes the acquisition of new mutations, which either in drug targets or the domains those active alternative pathways able to render cell survival[64].

4.2. *Modifications of gene copy number and expression level*

Gene copy number alteration and protein expression level change are another two major mechanisms of oncogenic activation or signaling pathway modification. MET amplification represents the strongest evidence that cells treated with TKIs tend to acquire genetic alterations to tolerate the inhibition. The MET gene encodes a transmembrane tyrosine kinase receptor that acts as an HGF receptor and is involved with invasion, metastasis, and angiogenesis in tumors[65]. Activation of MET has been shown to protect cancer cells from DNA damage[66]. Clinical evidences have indicated that amplification of the MET oncogene is observed in 20% of resistance cases in NSCLC patients with Gefitinib or Erlotinib treatment[67]. As a consequence, tumor cells undergo an adaptive process and acquire MET amplification during the selection, but not due to selection of a preexisting population of cells. Those results in receptor overexpression and ligand-independent activation featured as the concept of "oncogenic addiction"[68]. It is likely that cells gaining MET extra copies have a selective advantage under the selective pressure of the drug. In Gefitinib resistant HCC827 cells, a focal amplification is generated in chromosome 7 that harboring the MET oncogene[69]. However, FISH analysis shows that the acquired copies of MET do not located on chromosome 7 (where the MET gene is positioned) but on a marker chromosome[70]. This suggests a mechanism of progressive acquisition of additional MET copies as a consequence of asymmetric partitioning of the marker chromosome at mitosis[71]. Acquired resistance of NSCLC cells to TKIs is mainly mediated by a switch to EGFR dependency, which indicates a reciprocal and complementary relationship between T790M mutation and MET amplification[72,73]. Unfortunately, MET amplification often accompanies with EGFR amplification or KRAS amplification, which results in MET TKIs therapy failure[71,74].

On the other hand, hepatocyte growth factor (HGF) overexpression may lead to MET ligand-dependent activation. It is proved that the mechanism of intrinsic resistance to Gefitinib in NSCLC cells with EGFR-activating mutations is not MET-amplified[75]. Notably in some patients without evidence of EGFR T790M mutation or MET amplification, HGF expression is greater in the resistant specimen, supporting a role for HGF alone in promoting drug resistance[69]. It has been proposed that activation of HGF/MET signaling can lead to Gefitinib resistance in EGFR mutant cancers by activating PI3K/AKT signal pathway through two different adapters: ERBB3 when MET is activated by genomic amplification or GAB1 (Grb2 associated binder 1) when MET is activated by HGF[69,76].

Another well-described mechanism underlying clinical resistance to Imatinib is BCR–ABL gene amplification or increased mRNA levels of that. It is demonstrated that both are responsible for an increased level of protein, which is able to restore oncogenic signaling in presence of a given drug concentration[77]. Cytogenetic and molecular techniques, i.e., expression of BCR–ABL transcripts is quantified using the quantitative real-time PCR assay and BCR–ABL gene amplification is detected using fluorescence *in situ* hybridization, are currently used to monitor CML therapy for both response and relapse[78]. It is also possible that overexpression of BCR–ABL may be an early phenomenon, preceding the emergence of a dominant clone with a mutant kinase domain[79]. Of note, the mechanism underlying genomic amplification is likely due to the genomic instability. Additionally, maintenance of glucose uptake for cell metabolism can inhibit p53 activation and promote resistance when BCR–ABL-expressing cells were treated with Imatinib[80].

On the contrary, Virgili et al.[81] reported that loss of the remaining normal ABL1 allele in CML, which resulted from cryptic interstitial deletion in 9q34 in patients who did not achieve a complete cytogenetic remission (CCyR) during treatment, engenders a novel unexpected mechanism of Imatinib resistance. In addition, patients harbor deletion mutation on exon 19 of EGFR gene or in-frame deletion delE746-A750 follow T790M mutation or epithelial-to-mesenchymal transition (EMT), which is related with an acquired resistance to Gefitinib or Erlotinib[82,83].

The above results showed that these alterations in gene or protein expression could account for all resistant mechanisms. This phenomenon suggests the existence of complicated relationships among acquired resistance-related genes[24].

4.3. *Modification of signaling pathways*

Cancer cells can survival and replace the lack of signal in target therapy by activating modified signaling pathway, leading to the acquisition of drug resistance. EGFR-TKI, such as Gefitinib and Erlotinib, shows favorable response to EGFR mutant lung cancer. However, the responders may acquire resistance induced by HGF, which activates MET that restores downstream mitogen activated protein kinase (MAPK)/extracellular signal regulated kinase (ERK)1/2 and phosphoinositide 3-kinase (PI3K)/Akt signaling[84]. Mink et al.[85] provided evidence that paracrine factors secreted from the EGFR-TKI-resistant CAFs (cancer-associated fibroblast population) mitigate the EGFR-TKI-mediated blockade of pEGFR and pMAPK in co-cultured tumor cells, regardless of their EGFR mutational status. Additionally, elevated IGFR-1β phosphorylation can compensate for the loss of EGFR signaling function. Either increased insulin-like growth factor II expression induced by Gefitinib, or heterodimerization of EGFR and IGFR-1β, may trigger IGFR-1β signal transduction via activation of Akt and MAPK, and the crosstalk between EGFR and IGFR-1β signaling are likely to contribute to resistance of CRC cells to this agent[86]. Interestingly, Dumka et al.[87] raised the possibility that development of novel means to enhance p38 MAPK activation in BCR/ABL expressing cells may be an approach to promote antileukemic effects of Dasatinib and, possibly, reverse T315I mutation-mediated resistance. In the other way, Src kinase inhibition with Dasatinib seems to be related to a lack of inhibition of STAT3 and MAPK signaling[88]. In a similar manner, Suzuki et al.[89] reported a new mechanism of Imatinib resistance mediated by the activation of RAS/MAPK pathway and EphB4.

Moreover, several possible mechanisms of acquired EGFR-TKIs resistance, such as the involvement of insulin-like growth factor1 receptor (IGF1R) signaling, the loss of PTEN, or PI3K-dependent recruitment of Gab1/Shp2 overexpression, were reported[90]. PTEN instability-mediated constitutive Akt activation is involved in acquired resistance to cetuximab and also induces *de novo* resistance to Gefitinib[91]. Exposure of Imatinib-resistant EOL-1R cells, which showed epigenetic silencing of the phosphatase and PTEN gene, to Imatinib failed to dephosphorylate AKT, ERK and STAT5, although PDGFRa was effectively inactivated[92]. Another example is that PTEN inactivation specifically raises EGFR activity by impairing the ligand-induced ubiquitylation and degradation of the activated receptor through destabilization of newly formed ubiquitin ligase Cbl complexes[93]. However, loss of PTEN expression has not been found to be associated with Lapatinib resistance in any cell lines or clinical specimens[94]. When chronically exposing HER2-overexpressing cells to Lapatinib, resistant cells were found more dependent on estrogen receptor signaling in terms of cell survival than parent cells[95].

Another mechanism of resistance is that PI3K pathway inhibitors impaired dephosphorylation of RPS6 (the ribosomal S6 protein) in Imatinib-resistant cell lines, suggesting that an oncogene other than BCR–ABL1 might be responsible for activation of the PI3K/AKT1/mTOR pathway[96]. In another signaling pathway, hyper activation of the pharmacologically targetable PI3K/mTOR/p70S6K1 axis appears to be central to the occurrence of Lapatinib resistance in breast cancer[97]. In HCC, activation of PI3K/Akt signaling pathway mediates acquired resistance to Sorafenib therapy[98,99].

4.4. *Mechanisms of resistance related to drug influx/efflux*

Anticancer drug resistance, including TKIs, almost invariably emerges and poses major obstacles towards curative therapy of

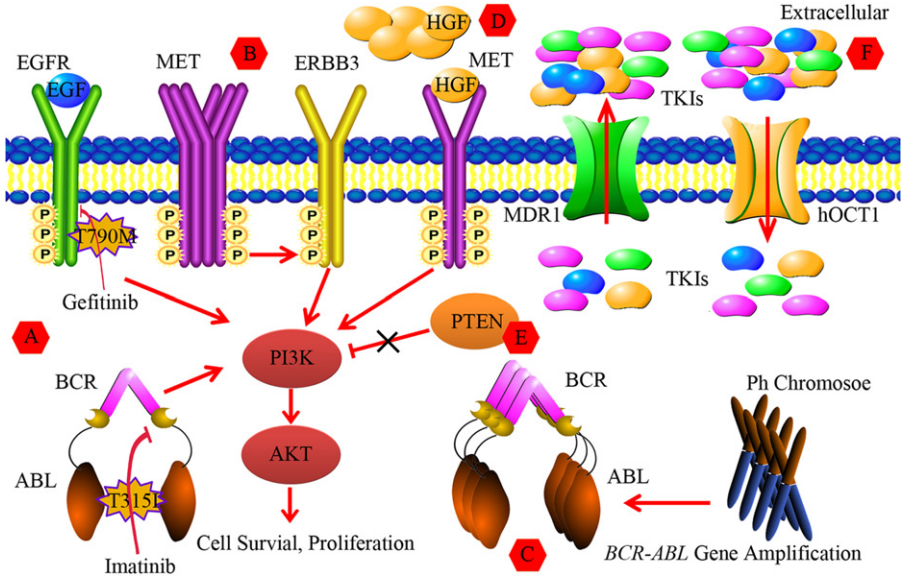


Figure 1 Schematic summary of the main molecular mechanism of acquired resistance to TKIs. (A) Mutations in the EGFR or BCR–ABL kinase domain, including T790M or T315I, can decrease or abolish the inhibitory effect of the drug; (B,C) Gene amplification, such as *MET* or *BCR–ABL,* leading to overproduction of the TK can confer relative resistance to an inhibitor; (D) Overexpression of RTK ligands mediated tumor cells activation without control; (E) Modification of signaling pathways, such as PTEN instability-mediated constitutive Akt activation; (F) Increased efflux or decreased influx of TKIs from the cancer cell, mediated by membrane transporters such as MDR1 or hOCT1, can decrease intracellular concentrations.

various human malignancies[100]. In tumor cell lines, multidrug resistance (MDR) is often associated with an ATP-dependent decrease in cellular drug accumulation, which is attributed to the overexpression of certain ATP-binding cassette (ABC) transporter proteins[101]. Among ABC transporters, overexpression of P-glycoprotein (MDR1/P-gp/ABCB1) and the breast cancer resistance protein (BCRP/ABCG2) confer resistance to Imatinib in CML or Gefitinib in NSCLN[102,103]. *In vitro* study showed that chronic Imatinib exposure of Caco-2 cells resulted in a ~50% decrease in intracellular accumulation of Imatinib, probably due to enhanced ABCG2- and MDR1-mediated efflux, as a result of upregulated expression of these drug pumps[104]. Further investigation indicated that not only Imatinib, Gefitinib, Tandutinib but Dasatinib are high-affinity substrates of MDR1 and ABCG2, This may explain why these proteins mediated an effective resistance in cancer cells against above compounds[105–108]. Recent evidences have established that both Sunitinib and Sorafenib are recognized and bound by both MDR1 and ABCG2, and can also be effluxed in a specific concentration window[109]. These finding illustrate that MDR1 and ABCG2 play roles in oral absorption, systemic clearance, and cell penetration of certain TKIs in patients. Collectively, overexpression of ABC transporters protects tumor cells from TKIs inhibition that the chemo-immune system seems to recognize targeted TKI drugs as xenobiotics at the membrane and tissue barriers and, in case of active extrusion, protects intracellular targets from the action of the TKIs[109]. And overexpression of drug transporters may allow the evolution of genetic alteration cells that confer more potential drug resistance.

Recently, another drug transporters, human organic cation transporter 1 (hOCT1), has been implicated as possible mechanism for promoting Imatinib resistance in CML[110]. Clinical data suggested that of patients with higher than median (high) hOCT1 activity, 85% achieved major molecular response (MMR) by 24 months, versus 45% with no more than a median (low) hOCT1 activity[111]. Whereas hOCT1-mediated influx may be a key determinant of molecular response to Imatinib, it is unlikely to impact on cellular uptake and patient response to Nilotinib[104,112]. In conclusion, differential expression of influx (hOCT1) and efflux (MDR1, ABCG2) transporters may be a critical determinant of intracellular drug levels and, hence, resistance to Imatinib[113].

In fact, TKIs such as Nilotinib, Lapatinib, Gefitinib and Erlotinib showed an effective outcome of reverse ABC transporters by blocking their efflux function[106,114-116]. Noguchi et al.[117] found that Erlotinib effectively suppressed MDR1-mediated resistance to vincristine and paclitaxel, but did not suppress resistance to mitoxantrone and doxorubicin. Conversely, Erlotinib appeared to enhance MDR1-mediated resistance to mitoxantrone in K562/MDR cells. Nevertheless, a better understanding of the pharmacological interactions of TKIs used in combinational chemotherapy is important when coadministration of transporter modulators.

5. Conclusions

So far, a lot of TKIs have been identified and approved for treatment of cancer. However, the responders acquire resistance almost without exception. Understanding mechanisms of resistance to TKIs and developing treatment strategies to overcome resistance are the most important in the current research. Moreover, clinical monitoring of mutations, certain proteins overexpression, or gene amplifications should allow loss response to TKIs to be recognized. In addition, quiescence, microenvironment or microRNA may play roles in mediating resistance to TKIs. Importantly, these results also underscore the notion that a single cancer can simultaneously develop resistance induced by several mechanisms (Fig. 1)[68]. On the other hand, the lessons learnt from the TKIs resistance can drive researchers to develop next generation of TKIs and to design highly effective individualized therapies for cancer patients.

References

1. Hegedus T, Orfi L, Seprodi A, Varadi A, Sarkadi B, Keri G. Interaction of tyrosine kinase inhibitors with the human multidrug transporter proteins, MDR1 and MRP1. *Biochim Biophys Acta* 2002;**1587**:318–25.
2. Yoshida T, Zhang G, Haura EB. Targeting epidermal growth factor receptor: central signaling kinase in lung cancer. *Biochem Pharmacol* 2010;**80**:613–23.
3. Grimminger F, Schermuly RT, Ghofrani HA. Targeting non-malignant disorders with tyrosine kinase inhibitors. *Nat Rev Drug Discov* 2010;**9**:956–70.
4. Manning G, Whyte DB, Martinez R, Hunter T, Sudarsanam S. The protein kinase complement of the human genome. *Science* 2002;**298**:1912–34.
5. Hunter T. The Croonian Lecture 1997. The phosphorylation of proteins on tyrosine: its role in cell growth and disease. *Philos Trans R Soc Lond B Biol Sci* 1998;**353**:583–605.
6. Lemmon MA, Schlessinger J. Cell signaling by receptor tyrosine kinases. *Cell* 2010;**141**:1117–34.
7. Schlessinger J. Cell signaling by receptor tyrosine kinases. *Cell* 2000;**103**:211–25.
8. Gschwind A, Fischer OM, Ullrich A. The discovery of receptor tyrosine kinases: targets for cancer therapy. *Nat Rev Cancer* 2004;**4**:361–70.
9. Krause DS, van Etten RA. Tyrosine kinases as targets for cancer therapy. *N Engl J Med* 2005;**353**:172–87.
10. Reiter A, Walz C, Cross NC. Tyrosine kinases as therapeutic targets in BCR–ABL negative chronic myeloproliferative disorders. *Curr Drug Targets* 2007;**8**:205–16.
11. Wong WS. Inhibitors of the tyrosine kinase signaling cascade for asthma. *Curr Opin Pharmacol* 2005;**5**:264–71.
12. Sierra JR, Cepero V, Giordano S. Molecular mechanisms of acquired resistance to tyrosine kinase targeted therapy. Mol Cancer 2010; doi:10.1186/1476-4598-9-75.
13. Wang XK, Fu LW. Interaction of tyrosine kinase inhibitors with the MDR-related ABC transporter proteins. *Curr Drug Metab* 2010;**11**:618–28.
14. Chari RV. Targeted cancer therapy: conferring specificity to cytotoxic drugs. *Acc Chem Res* 2008;**41**:98–107.
15. Arora A, Scholar EM. Role of tyrosine kinase inhibitors in cancer therapy. *J Pharmacol Exp Ther* 2005;**315**:971–9.
16. Shawver LK, Slamon D, Ullrich A. Smart drugs: tyrosine kinase inhibitors in cancer therapy. *Cancer Cell* 2002;**1**:117–23.
17. Ocana A, Pandiella A, Siu LL, Tannock IF. Preclinical development of molecular-targeted agents for cancer. *Nat Rev Clin Oncol* 2011;**8**:200–9.
18. Hanfstein B, Muller MC, Kreil S, Ernst T, Schenk T, Lorentz C, et al. Dynamics of mutant BCR–ABL-positive clones after cessation of tyrosine kinase inhibitor therapy. *Haematologica* 2011;**96**:360–6.
19. Tanaka R, Kimura S. Abl tyrosine kinase inhibitors for overriding Bcr–Abl/T315I: from the second to third generation. *Expert Rev Anticancer Ther* 2008;**8**:1387–98.
20. Suda K, Onozato R, Yatabe Y, Mitsudomi T. EGFR T790M mutation: a double role in lung cancer cell survival? *J Thorac Oncol* 2009;**4**:1–4

21. Yun CH, Boggon TJ, Li Y, Woo MS, Greulich H, Meyerson M, et al. Structures of lung cancer-derived EGFR mutants and inhibitor complexes: mechanism of activation and insights into differential inhibitor sensitivity. *Cancer Cell* 2007;**11**:217–27.
22. Godin-Heymann N, Bryant I, Rivera MN, Ulkus L, Bell DW, Riese 2nd DJ, et al. Oncogenic activity of epidermal growth factor receptor kinase mutant alleles is enhanced by the T790M drug resistance mutation. *Cancer Res* 2007;**67**:7319–26.
23. Rho JK, Choi YJ, Jeon BS, Choi SJ, Cheon GJ, Woo SK, et al. Combined treatment with silibinin and epidermal growth factor receptor tyrosine kinase inhibitors overcomes drug resistance caused by T790M mutation. *Mol Cancer Ther* 2010;**9**:3233–43.
24. Uramoto H, Shimokawa H, Hanagiri T, Kuwano M, Ono M. Expression of selected gene for acquired drug resistance to EGFR-TKI in lung adenocarcinoma. *Lung Cancer* 2011;**73**:361–5.
25. Oxnard GR, Arcila ME, Sima CS, Riely GJ, Chmielecki J, Kris MG, et al. Acquired resistance to EGFR tyrosine kinase inhibitors in EGFR-mutant lung cancer: distinct natural history of patients with tumors harboring the T790M mutation. *Clin Cancer Res* 2011;**17**:1616–22.
26. Yamada T, Matsumoto K, Wang W, Li Q, Nishioka Y, Sekido Y, et al. Hepatocyte growth factor reduces susceptibility to an irreversible epidermal growth factor receptor inhibitor in EGFR-T790M mutant lung cancer. *Clin Cancer Res* 2010;**16**:174–83.
27. Manrique AG, Scholl V, Perez V, Bittencourt R, Moellmann A, Hassan R, et al. Rapid and sensitive allele-specific (AS)-RT-PCR assay for detection of T315I mutation in chronic myeloid leukemia patients treated with tyrosine-kinase inhibitors. *Clin Exp Med* 2011;**11**:55–9.
28. Kim D, Kim DW, Cho BS, Goh HG, Kim SH, Kim WS, et al. Structural modeling of V299L and E459K Bcr–Abl mutation, and sequential therapy of tyrosine kinase inhibitors for the compound mutations. *Leuk Res* 2009;**33**:1260–5.
29. Regales L, Balak MN, Gong Y, Politi K, Sawai A, Le C, et al. Development of new mouse lung tumor models expressing EGFR T790M mutants associated with clinical resistance to kinase inhibitors. *PLoS One* 2007;**2**:e810.
30. Alexandra EEB, Kenneth KI, John DH, Mark RM. Mechanisms of resistance to EGFR tyrosine kinase inhibitors:implications for patient selection and drug combination strategies. *Targeted Oncology* 2008;**3**:9.
31. Settleman J, Kurie JM. Drugging the bad ''AKT-TOR'' to overcome TKI-resistant lung cancer. *Cancer Cell* 2007;**12**: 6–8.
32. Mulloy R, Ferrand A, Kim Y, Sordella R, Bell DW, Haber DA, et al. Epidermal growth factor receptor mutants from human lung cancers exhibit enhanced catalytic activity and increased sensitivity to gefitinib. *Cancer Res* 2007;**67**:2325–30.
33. Sharma SV, Bell DW, Settleman J, Haber DA. Epidermal growth factor receptor mutations in lung cancer. *Nat Rev Cancer* 2007;**7**: 169–81.
34. Burke AC, Swords RT, Kelly K, Giles FJ. Current status of agents active against the T315I chronic myeloid leukemia phenotype. *Expert Opin Emerg Drugs* 2011;**16**:85–103.
35. Hughes TP, Branford S. Monitoring disease response to tyrosine kinase inhibitor therapy in CML. *Hematology Am Soc Hematol Educ Program* 2009:477–87.
36. La Rosee P, Deininger MW. Resistance to imatinib: mutations and beyond. *Semin Hematol* 2010;**47**:335–43.
37. Apperley JF, Part I. Mechanisms of resistance to imatinib in chronic myeloid leukaemia. *Lancet Oncol* 2007;**8**:1018–29.
38. Mancini M, Corradi V, Petta S, Barbieri E, Manetti F, Botta M, et al. A new nonpeptidic inhibitor of 14-3-3 induces apoptotic cell death in chronic myeloid leukemia sensitive or resistant to imatinib. *J Pharmacol Exp Ther* 2011;**336**:596–604.
39. Agrawal M, Garg RJ, Kantarjian H, Cortes J. Chronic myeloid leukemia in the tyrosine kinase inhibitor era: what is the ''best'' therapy? *Curr Oncol Rep* 2010;**12**:302–13
40. Liegl B, Kepten I, Le C, Zhu M, Demetri GD, Heinrich MC, et al. Heterogeneity of kinase inhibitor resistance mechanisms in GIST. *J Pathol* 2008;**216**:64–74.
41. Cassier PA, Blay JY. Molecular response prediction in gastrointestinal stromal tumors. *Target Oncol* 2010;**5**:29–37.
42. Corless CL, Fletcher JA, Heinrich MC. Biology of gastrointestinal stromal tumors. *J Clin Oncol* 2004;**22**:3813–25.
43. Corless CL, Schroeder A, Griffith D, Town A, McGreevey L, Harrell P, et al. PDGFRA mutations in gastrointestinal stromal tumors: frequency, spectrum and *in vitro* sensitivity to imatinib. *J Clin Oncol* 2005;**23**:5357–64.
44. Mol CD, Fabbro D, Hosfield DJ. Structural insights into the conformational selectivity of STI-571 and related kinase inhibitors. *Curr Opin Drug Discov Devel* 2004;**7**:639–48.
45. Gajiwala KS, Wu JC, Christensen J, Deshmukh GD, Diehl W, DiNitto JP, et al. KIT kinase mutants show unique mechanisms of drug resistance to imatinib and sunitinib in gastrointestinal stromal tumor patients. *Proc Natl Acad Sci USA* 2009;**106**:1542–7.
46. Sun J, Pedersen M, Ronnstrand L. The D816V mutation of c-Kit circumvents a requirement for Src family kinases in c-Kit signal transduction. *J Biol Chem* 2009;**284**:11039–47.
47. Antonescu CR, Busam KJ, Francone TD, Wong GC, Guo T, Agaram NP, et al. L576P KIT mutation in anal melanomas correlates with KIT protein expression and is sensitive to specific kinase inhibition. *Int J Cancer* 2007;**121**:257–64.
48. Prenen H, Cools J, Mentens N, Folens C, Sciot R, Schoffski P, et al. Efficacy of the kinase inhibitor SU11248 against gastrointestinal stromal tumor mutants refractory to imatinib mesylate. *Clin Cancer Res* 2006;**12**:2622–7.
49. Sjolund K, Andersson A, Nilsson E, Nilsson O, Ahlman H, Nilsson B. Downsizing treatment with tyrosine kinase inhibitors in patients with advanced gastrointestinal stromal tumors improved resectability. *World J Surg* 2010;**34**:2090–7.
50. Pratz KW, Sato T, Murphy KM, Stine A, Rajkhowa T, Levis M. FLT3-mutant allelic burden and clinical status are predictive of response to FLT3 inhibitors in AML. *Blood* 2010;**115**:1425–32.
51. Stirewalt DL, Meshinchi S. Receptor tyrosine kinase alterations in AML – biology and therapy. *Cancer Treat Res* 2010;**145**:85–108.
52. Youssoufian H, Rowinsky EK, Tonra J, Li Y. Targeting FMS-related tyrosine kinase receptor 3 with the human immunoglobulin G1 monoclonal antibody IMC-EB10. *Cancer* 2010;**116**: 1013–7.
53. Piloto O, Wright M, Brown P, Kim KT, Levis M, Small D. Prolonged exposure to FLT3 inhibitors leads to resistance via activation of parallel signaling pathways. *Blood* 2007;**109**:1643–52.
54. Heidel F, Solem FK, Breitenbuecher F, Lipka DB, Kasper S, Thiede MH, et al. Clinical resistance to the kinase inhibitor PKC412 in acute myeloid leukemia by mutation of Asn-676 in the FLT3 tyrosine kinase domain. *Blood* 2006;**107**:293–300.
55. Weisberg E, Sattler M, Ray A, Griffin JD. Drug resistance in mutant FLT3-positive AML. *Oncogene* 2010;**29**:5120–34.
56. von Bubnoff N, Engh RA, Aberg E, Sanger J, Peschel C, Duyster J. FMS-like tyrosine kinase 3-internal tandem duplication tyrosine kinase inhibitors display a nonoverlapping profile of resistance mutations *in vitro*. *Cancer Res* 2009;**69**:3032–41.
57. Frohling S, Scholl C, Levine RL, Loriaux M, Boggon TJ, Bernard OA, et al. Identification of driver and passenger mutations of FLT3 by high-throughput DNA sequence analysis and functional assessment of candidate alleles. *Cancer Cell* 2007;**12**:501–13.
58. Bagrintseva K, Schwab R, Kohl TM, Schnittger S, Eichenlaub S, Ellwart JW, et al. Mutations in the tyrosine kinase domain of FLT3 define a new molecular mechanism of acquired drug resistance to PTK inhibitors in FLT3-ITD-transformed hematopoietic cells. *Blood* 2004;**103**:2266–75.
59. Cools J, Mentens N, Furet P, Fabbro D, Clark JJ, Griffin JD, et al. Prediction of resistance to small molecule FLT3 inhibitors: implications for molecularly targeted therapy of acute leukemia. *Cancer Res* 2004;**64**:6385–9.

60. Quentmeier H, Eberth S, Romani J, Zaborski M. Drexler HG. BCR–ABL1-independent PI3Kinase activation causing imatinib-resistance. J Hematol Oncol 2011; doi:10.1186/1756-8722-4-6.
61. Horn S, Bergholz U, Jucker M, McCubrey JA, Trumper L. Stocking C, et al. Mutations in the catalytic subunit of class IA PI3K confer leukemogenic potential to hematopoietic cells. *Oncogene* 2008;**27**:4096–106.
62. Tiseo M, Gelsomino F, Boggiani D, Bortesi B, Bartolotti M, Bozzetti C, et al. EGFR and EML4-ALK gene mutations in NSCLC: a case report of erlotinib-resistant patient with both concomitant mutations. *Lung Cancer* 2011;**71**:241–3.
63. Costa DB, Halmos B, Kumar A, Schumer ST, Huberman MS, Boggon TJ, et al. BIM mediates EGFR tyrosine kinase inhibitor-induced apoptosis in lung cancers with oncogenic EGFR mutations. *PLoS Med* 2007;**4**:1669–79.
64. Nowak D, Ogawa S, Muschen M, Kato M, Kawamata N, Meixel A, et al. SNP array analysis of tyrosine kinase inhibitor-resistant chronic myeloid leukemia identifies heterogeneous secondary genomic alterations. *Blood* 2010;**115**:1049–53.
65. Kosaka T, Yamaki E, Mogi A, Kuwano H. Mechanisms of resistance to EGFR TKIs and development of a new generation of drugs in non-small-cell lung cancer. *J Biomed Biotechnol* 2011; doi:10.1155/2011/165214.
66. Welsh JW, Mahadevan D, Ellsworth R, Cooke L, Bearss D, Stea B. The c-Met receptor tyrosine kinase inhibitor MP470 radiosensitizes glioblastoma cells. *Radiat Oncol* 2009; doi:10.1186/1748-717X-4-69.
67. Bean J, Brennan C, Shih JY, Riely G, Viale A, Wang L, et al. MET amplification occurs with or without T790M mutations in EGFR mutant lung tumors with acquired resistance to gefitinib or erlotinib. *Proc Natl Acad Sci USA* 2007;**104**:20932–7.
68. Qi J, McTigue MA, Rogers A, Lifshits E, Christensen JG, Janne PA, et al. Multiple mutations and bypass mechanisms can contribute to development of acquired resistance to MET inhibitors. *Cancer Res* 2011;**71**:1081–91.
69. Turke AB, Zejnullahu K, Wu YL, Song Y, Dias-Santagata D, Lifshits E, et al. Preexistence and clonal selection of MET amplification in EGFR mutant NSCLC. *Cancer Cell* 2010;**17**:77–88.
70. Corso S, Ghiso E, Cepero V, Sierra JR, Migliore C, Bertotti A, et al. Activation of HER family members in gastric carcinoma cells mediates resistance to MET inhibition. *Mol Cancer* 2010; doi:10.1186/1476-4598-9-121.
71. Cepero V, Sierra JR, Corso S, Ghiso E, Casorzo L, Perera T, et al. MET and KRAS gene amplification mediates acquired resistance to MET tyrosine kinase inhibitors. *Cancer Res* 2010;**70**:7580–90.
72. McDermott U, Pusapati RV, Christensen JG, Gray NS, Settleman J. Acquired resistance of non-small cell lung cancer cells to MET kinase inhibition is mediated by a switch to epidermal growth factor receptor dependency. *Cancer Res* 2010;**70**:1625–34.
73. Suda K, Murakami I, Katayama T, Tomizawa K, Osada H, Sekido Y, et al. Reciprocal and complementary role of MET amplification and EGFR T790M mutation in acquired resistance to kinase inhibitors in lung cancer. *Clin Cancer Res* 2010;**16**: 5489–98.
74. Sequist LV, Waltman BA, Dias-Santagata D, Digumarthy S, Turke AB, Fidias P, et al. Genotypic and histological evolution of lung cancers acquiring resistance to EGFR inhibitors. *Sci Transl Med* 2011; doi:10.1126/scitranslmed.3002003.
75. Liska D, Chen CT, Bachleitner-Hofmann T, Christensen JG, Weiser MR. HGF rescues colorectal cancer cells from EGFR inhibition via MET activation. *Clin Cancer Res* 2011;**17**:472–82.
76. Nam HJ, Chae S, Jang SH, Cho H, Lee JH. The PI3K-Akt mediates oncogenic Met-induced centrosome amplification and chromosome instability. *Carcinogenesis* 2010;**31**:1531–40.
77. Volpe G, Panuzzo C, Ulisciani S, Cilloni D. Imatinib resistance in CML. *Cancer Lett* 2009;**274**:1–9.
78. Sharma P, Mohanty S, Kochupillai V, Kumar L. Mutations in ABL kinase domain are associated with inferior progression-free survival. *Leuk Lymphoma* 2010;**51**:1072–8.
79. Breccia M, Alimena G. Resistance to imatinib in chronic myeloid leukemia and therapeutic approaches to circumvent the problem. *Cardiovasc Hematol Disord Drug Targets* 2009;**9**:21–8.
80. Mason EF, Zhao Y, Goraksha-Hicks P, Coloff JL, Gannon H, Jones SN, et al. Aerobic glycolysis suppresses p53 activity to provide selective protection from apoptosis upon loss of growth signals or inhibition of BCR–Abl. *Cancer Res* 2010;**70**:8066–76.
81. Virgili A, Koptyra M, Dasgupta Y, Glodkowska-Mrowka E, Stoklosa T, Nacheva EP, et al. Imatinib sensitivity in BCR–ABL1-positive chronic myeloid leukemia cells is regulated by the remaining normal ABL1 allele. *Cancer Res* 2011;**71**:5381–6.
82. Chung JH, Rho JK, Xu X, Lee JS, Yoon HI, Lee CT, et al. Clinical and molecular evidences of epithelial to mesenchymal transition in acquired resistance to EGFR-TKIs. *Lung Cancer* 2011;**73**:176–82.
83. Kwak EL, Jankowski J, Thayer SP, Lauwers GY, Brannigan BW, Harris PL, et al. Epidermal growth factor receptor kinase domain mutations in esophageal and pancreatic adenocarcinomas. *Clin Cancer Res* 2006;**12**:4283–7.
84. Donev IS, Wang W, Yamada T, Li Q, Takeuchi S, Matsumoto K, et al. Transient PI3K inhibition induces apoptosis and overcomes HGF-mediated resistance to EGFR-TKIs in EGFR mutant lung cancer. *Clin Cancer Res* 2011;**17**:2260–9.
85. Mink SR, Vashistha S, Zhang W, Hodge A, Agus DB, Jain A. Cancer-associated fibroblasts derived from EGFR-TKI-resistant tumors reverse EGFR pathway inhibition by EGFR-TKIs. *Mol Cancer Res* 2010;**8**:809–20.
86. Yang L, Li J, Ran L, Pan F, Zhao X, Ding Z, et al. Phosphorylated insulin-like growth factor 1 receptor is implicated in resistance to the cytostatic effect of gefitinib in colorectal cancer cells. *J Gastrointest Surg* 2011;**15**:942–57.
87. Dumka D, Puri P, Carayol N, Lumby C, Balachandran H, Schuster K, et al. Activation of the p38 Map kinase pathway is essential for the antileukemic effects of dasatinib. *Leuk Lymphoma* 2009;**50**:2017–29.
88. Nagaraj NS, Smith JJ, Revetta F, Washington MK, Merchant NB. Targeted inhibition of SRC kinase signaling attenuates pancreatic tumorigenesis. *Mol Cancer Ther* 2010;**9**:2322–32.
89. Suzuki M, Abe A, Imagama S, Nomura Y, Tanizaki R, Minami Y, et al. BCR–ABL-independent and RAS/MAPK pathway-dependent form of imatinib resistance in Ph-positive acute lymphoblastic leukemia cell line with activation of EphB4. *Eur J Haematol* 2010;**84**:229–38.
90. Carella AM. Hypothesis: upfront use of ABL kinase inhibitor combination, either simultaneously or sequentially, in high-risk Ph+ leukemias? *Ann Hematol* 2010;**89**:531–3
91. Kim SM, Kim JS, Kim JH, Yun CO, Kim EM, Kim HK, et al. Acquired resistance to cetuximab is mediated by increased PTEN instability and leads cross-resistance to gefitinib in HCC827 NSCLC cells. *Cancer Lett* 2010;**296**:150–9.
92. Nishioka C, Ikezoe T, Yang J, Yokoyama A. Long-term exposure of leukemia cells to multi-targeted tyrosine kinase inhibitor induces activations of AKT, ERK and STAT5 signaling via epigenetic silencing of the PTEN gene. *Leukemia* 2010;**24**:1631–40.
93. Vivanco I, Rohle D, Versele M, Iwanami A, Kuga D, Oldrini B, et al. The phosphatase and tensin homolog regulates epidermal growth factor receptor (EGFR) inhibitor response by targeting EGFR for degradation. *Proc Natl Acad Sci USA* 2010;**107**: 6459–64.
94. Mukohara T. Mechanisms of resistance to anti-human epidermal growth factor receptor 2 agents in breast cancer. *Cancer Sci* 2011;**102**:1–8.
95. Xia W, Bacus S, Hegde P, Husain I, Strum J, Liu L, et al. A model of acquired autoresistance to a potent ErbB2 tyrosine

kinase inhibitor and a therapeutic strategy to prevent its onset in breast cancer. *Proc Natl Acad Sci USA* 2006;**103**:7795–800.
96. Morgillo F, D'Aiuto E, Troiani T, Martinelli E, Cascone T, de Palma R, et al. Antitumor activity of bortezomib in human cancer cells with acquired resistance to anti-epidermal growth factor receptor tyrosine kinase inhibitors. *Lung Cancer* 2011;**71**: 283–90.
97. Yang X, Zhang X, Yang J, Huang Y, Guo A, Lin J, et al. Analysis of differentially expressed proteins in self-paired sera of advanced non-small cell lung cancer patients responsive to Gefinitib. *Zhongguo Fei Ai Za Zhi* 2009;**12**:765–9.
98. Chen KF, Chen HL, Tai WT, Feng WC, Hsu CH, Chen PJ, et al. Activation of phosphatidylinositol 3-kinase/Akt signaling pathway mediates acquired resistance to sorafenib in hepatocellular carcinoma cells. *J Pharmacol Exp Ther* 2011;**337**:155–61.
99. Ichihara E, Ohashi K, Takigawa N, Osawa M, Ogino A, Tanimoto M, et al. Effects of vandetanib on lung adenocarcinoma cells harboring epidermal growth factor receptor T790M mutation *in vivo*. *Cancer Res* 2009;**69**:5091–8.
100. Jabbour E, Cortes J, Kantarjian H. Long-term outcomes in the second-line treatment of chronic myeloid leukemia: a review of tyrosine kinase inhibitors. *Cancer* 2011;**117**:897–906.
101. Leslie EM, Deeley RG, Cole SP. Multidrug resistance proteins: role of P-glycoprotein, MRP1, MRP2, and BCRP (ABCG2) in tissue defense. *Toxicol Appl Pharmacol* 2005;**204**:216–37.
102. Vasconcelos FC, Silva KL, de Souza PS, Silva LF, Moellmann-Coelho A, Klumb CE, et al. Variation of MDR proteins expression and activity levels according to clinical status and evolution of CML patients. *Cytometry B Clin Cytom* 2011;**80**: 158–66.
103. Azzariti A, Porcelli L, Simone GM, Quatrale AE, Colabufo NA, Berardi F, et al. Tyrosine kinase inhibitors and multidrug resistance proteins: interactions and biological consequences. *Cancer Chemother Pharmacol* 2010;**65**:335–46.
104. Davies A, Jordanides NE, Giannoudis A, Lucas CM, Hatziieremia S, Harris RJ, et al. Nilotinib concentration in cell lines and primary CD34(+) chronic myeloid leukemia cells is not mediated by active uptake or efflux by major drug transporters. *Leukemia* 2009;**23**: 1999–2006.
105. Graber-Maier A, Gutmann H, Drewe J. A new intestinal cell culture model to discriminate the relative contribution of P-gp and BCRP on transport of substrates such as Imatinib. *Mol Pharm* 2010; doi:10.1021/mp100040f.
106. Carcaboso AM, Elmeliegy MA, Shen J, Juel SJ, Zhang ZM. Calabrese C, et al. Tyrosine kinase inhibitor gefitinib enhances topotecan penetration of gliomas. *Cancer Res* 2010;**70**:4499–508.
107. Hegedus C, Ozvegy-Laczka C, Apati A, Magocsi M, Nemet K, Orfi L, et al. Interaction of nilotinib, dasatinib and bosutinib with ABCB1 and ABCG2: implications for altered anti-cancer effects and pharmacological properties. *Br J Pharmacol* 2009;**158**:1153–64.
108. Yang JJ, Milton MN, Yu S, Liao M, Liu N, Wu JT, et al. P-glycoprotein and breast cancer resistance protein affect disposition of tandutinib, a tyrosine kinase inhibitor. *Drug Metab Lett* 2010;**4**:201–12.
109. Brozik A, Hegedus C, Erdei Z, Hegedus T, Ozvegy-Laczka C, Szakacs G, et al. Tyrosine kinase inhibitors as modulators of ATP binding cassette multidrug transporters: substrates, chemosensitizers or inducers of acquired multidrug resistance? *Expert Opin Drug Metab Toxicol* 2011;**7**:623–42
110. Melo JV, Chuah C. Resistance to imatinib mesylate in chronic myeloid leukaemia. *Cancer Lett* 2007;**249**:121–32.
111. White DL, Saunders VA, Dang P, Engler J, Venables A, Zrim S, et al. Most CML patients who have a suboptimal response to imatinib have low OCT-1 activity: higher doses of imatinib may overcome the negative impact of low OCT-1 activity. *Blood* 2007;**110**:4064–72.
112. White DL, Saunders VA, Dang P, Engler J, Zannettino AC, Cambareri AC, et al. OCT-1-mediated influx is a key determinant of the intracellular uptake of imatinib but not nilotinib (AMN107): reduced OCT-1 activity is the cause of low *in vitro* sensitivity to imatinib. *Blood* 2006;**108**:697–704.
113. Thomas J, Wang L, Clark RE, Pirmohamed M. Active transport of imatinib into and out of cells: implications for drug resistance. *Blood* 2004;**104**:3739–45.
114. Kuang YH, Shen T, Chen X, Sodani K, Hopper-Borge E, Tiwari AK, et al. Lapatinib and erlotinib are potent reversal agents for MRP7 (ABCC10)-mediated multidrug resistance. *Biochem Pharmacol* 2010;**79**:154–61.
115. Dai CL, Tiwari AK, Wu CP, Su XD, Wang SR, Liu DG, et al. Lapatinib (Tykerb, GW572016) reverses multidrug resistance in cancer cells by inhibiting the activity of ATP-binding cassette subfamily B member 1 and G member 2. *Cancer Res* 2008;**68**: 7905–14.
116. Tiwari AK, Sodani K, Wang SR, Kuang YH, Ashby Jr. CR, Chen X, et al. Nilotinib (AMN107, Tasigna) reverses multidrug resistance by inhibiting the activity of the ABCB1/Pgp and ABCG2/BCRP/MXR transporters. *Biochem Pharmacol* 2009;**78**: 153–61.
117. Noguchi K, Kawahara H, Kaji A, Katayama K, Mitsuhashi J, Sugimoto Y. Substrate-dependent bidirectional modulation of P-glycoprotein-mediated drug resistance by erlotinib. *Cancer Sci* 2009;**100**:1701–7.