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Mechanisms of resistance to small molecule kinase inhibition in the treatment of solid tumors

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A growing number of tumors are characterized by simple genetic changes that activate important biochemical pathways, which are involved in their pathogenesis. These findings have led to the concept of targeted small molecule inhibitor treatment. The prototype for this type of therapy has been treatment of chronic myelogenous leukemia with imatinib mesylate (Gleevec), which targets BCR-ABL kinase. More recently, imatinib has been used to inhibit KIT in gastrointestinal (GI) stromal tumor, a mesenchymal tumor that arises in the GI tract. Furthermore, it has been possible to target EGFR in non-small-cell lung cancer with gefitinib and erlotinib. While initial results have been encouraging, resistance to small molecule kinase inhibitors is a substantial drawback. This paper focuses on what is known about mechanisms of resistance in the treatment of solid tumors by small molecule kinase inhibitors.
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Only recently, have we fully appreciated that a subset of cancers can be largely dependent on a single oncogenic pathway. This has led to the oncogene addiction hypothesis.[1] In a formal test of this hypothesis, Druker *et al*[2] proposed that inhibition of ABL-kinase might inhibit the growth of chronic myelogenous leukemia (CML), which is characterized by the BCR-ABL fusion oncoprotein in more than 90% of the cases. This strategy has been very successful as imatinib mesylate (STI-571, Gleevec/Glivec, Novartis Pharmaceuticals, Basel, Switzerland), a small molecule inhibitor of ABL kinase, has become the treatment of choice for BCR-ABL positive CML.[3] These results have been extended to solid tumors by targeting KIT in gastrointestinal stromal tumors (GIST),[4] and epidermal growth factor receptor (EGFR) in non-small-cell lung cancer (NSCLC).[5] Unfortunately, while these therapies have been successful initially, it has become clear that monotherapies designed to inhibit oncogenic kinases are unable to completely eradicate these tumors due to the development of resistance. This review summarizes the current state of what is known about mechanisms of resistance to small molecule inhibitors of oncogenic kinases in solid tumors.

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Background

GIST is characterized by the presence of oncogenic *KIT* or platelet-derived growth factor receptor alpha (*PDGFRA*) mutations.[6] KIT and PDGFRA are receptor tyrosine kinases with important roles in development, proliferation and cell death of many normal cell types, including interstitial cells of Cajal, the putative precursors to GIST. Mutations cluster within several exons of *KIT* (exons 9, 11, 13, and 17) and *PDGFRA* (exons 12, 14, and 18), preserve the open reading frame and result in ligand independent, constitutive activation of the kinase. These mutations are found within sporadic GISTs and also in rare familial GIST patients.[6] Furthermore, mice harboring corresponding mutations in their germ

line develop GISTs.[8,9] Imatinib mesylate, also potently inhibits KIT and PDGFRA, which led to its rapid FDA approval for treatment of metastatic GISTs after having shown considerable promise in clinical trials.

Approximately, 10% of NSCLCs are characterized by mutations in *EGFR* (epidermal growth factor receptor), a receptor tyrosine kinase with important roles in normal and malignant cell growth.[5] Within this group, non-smoking women of East Asian background with adenocarcinoma are overrepresented.[10] Mutations cluster within the ATP binding pocket in exons 18–21 of *EGFR*. As with GIST, these mutations preserve the open reading frame and encode constitutively active kinases. NSCLCs harboring these mutations are sensitive to the EGFR inhibitors gefitinib (Iressa, AstraZeneca, Wilmington, DE, USA) and erlotinib (Tarceva, OSI Pharmaceuticals, Melville, NY, USA).[11]

Primary resistance

Primary resistance refers to patients who either do not achieve stable disease or who progress within 6 months after an initial clinical response. In GIST, primary resistance to imatinib is seen predominantly in tumors with a *KIT* exon 9 mutation, most *PDGFRA* mutations, or no detectable kinase mutation (*KIT* and *PDGFRA* wild-type tumors).[12,13] At this point, the mechanism for primary resistance due to exon 9 mutations is unknown. However, there is some evidence that increasing the dose of imatinib might be able to overcome this resistance.[14] In contrast to *KIT* mutations, primary *PDGFRA* mutations often affect the kinase domain, presumably leading to conformational changes that confer primary imatinib resistance.[15]

Similar to what is seen in GIST without *KIT* or *PDGFRA* mutations, NSCLC without *EGFR* mutations are also refractory to treatment with EGFR inhibitors.[11] *K-ras* mutations are found in approximately 30% of NSCLC and these tumors do not contain *EGFR* mutations.[16] K-ras, is known to be a downstream signaling molecule in the EGFR pathway. Tumors harboring *K-ras* mutations appear to be insensitive to therapy with EGFR inhibitors.[16]

Secondary resistance

Secondary resistance to small molecule inhibition typically occurs after prolonged treatment, and several molecular mechanisms have been suggested to contribute to the resistance phenotype. However, only a few have been experimentally proven. The most common mechanism leads to structural alterations in the kinase domain of the originally affected kinase. This results in the inability of the inhibitor to bind to and inhibit the catalytic activity of the kinase. The primary kinase mutation is always maintained.

Secondary mutations in the kinase domain of *KIT* or *EGFR* arise in approximately 50% of patients with GIST or NSCLC that have acquired clinical resistance to imatinib or gefitinib/erlotinib therapy, respectively.[12,17–24]

Different regions of the kinase domain are affected by secondary mutations, and much of what has been described in imatinib-resistant CML also applies to GIST and NSCLC. A common mutation affects the so-called gatekeeper residue of the *BCR-ABL* fusion kinase, which is conserved in *KIT* and *EGFR* and is also found to be mutated in GIST and NSCLC, respectively (see Figure 1).[25] The gatekeeper residue consists of a threonine outside the actual catalytic core of the kinase. It is important for high-affinity binding to the inhibitor with which it forms a critical hydrogen bond. Most mutations at this site are predicted to abolish the ability to form this hydrogen bond. Additionally, a larger amino-acid side chain is introduced, leading to sterical hindrance.[25] Threonine to methionine mutations of the gatekeeper residue of *EGFR* (T790M) were seen in all but one NSCLC that developed resistance to gefitinib to date.[22–24] In GIST, the gatekeeper residue is mutated in about 20% of cases with a known resistance mutation and typically leads to substitutions of the original threonine by isoleucine (T670I) or phenylalanine (T670F).[12,17,20,21] This codon is located in a region (exon 14) where no primary *KIT* mutations have been found so far.

The most common secondary mutations in GISTs are found in *KIT* exons 13 and 17, both with a frequency of approximately 40%.[12,18–21] These regions correspond to the ATP-binding and phosphotransferase domains, also referred to as p-loop and activation loop. Similar mutations have been detected in CML patients, but are less common. These mutations presumably destabilize the closed (or inactive) conformation of the kinase and remove hydrophobic contacts.[25,26] As imatinib preferentially binds the inactive conformation of KIT, this is a potential 'Achilles' heel' that the cancers have exploited to develop resistance.[25] While the p-loop mutation in GIST is always a valine to alanine substitution at residue 654 (V654A), which is never seen in primary GIST, exon 17 mutations are variable. They cluster around codon 820 (816–823) and have been found in primary GISTs. Of note, a mutation involving *KIT* codon 816 (D816V) is also known to be associated with mast cell neoplasms and is resistant to imatinib inhibition.[27]

No secondary *PDGFRA* mutations have been described to date in GISTs that present with a primary *PDGFRA* mutation. The reason for this might lie in the fact that these mutations are less common and that *PDGFRA* mutations more frequently show primary resistance to imatinib.

Another mechanism that can also contribute to the development of resistance is generically known as 'kinase switch'.[28] This mechanism, in which tumor cells activate a different kinase than the

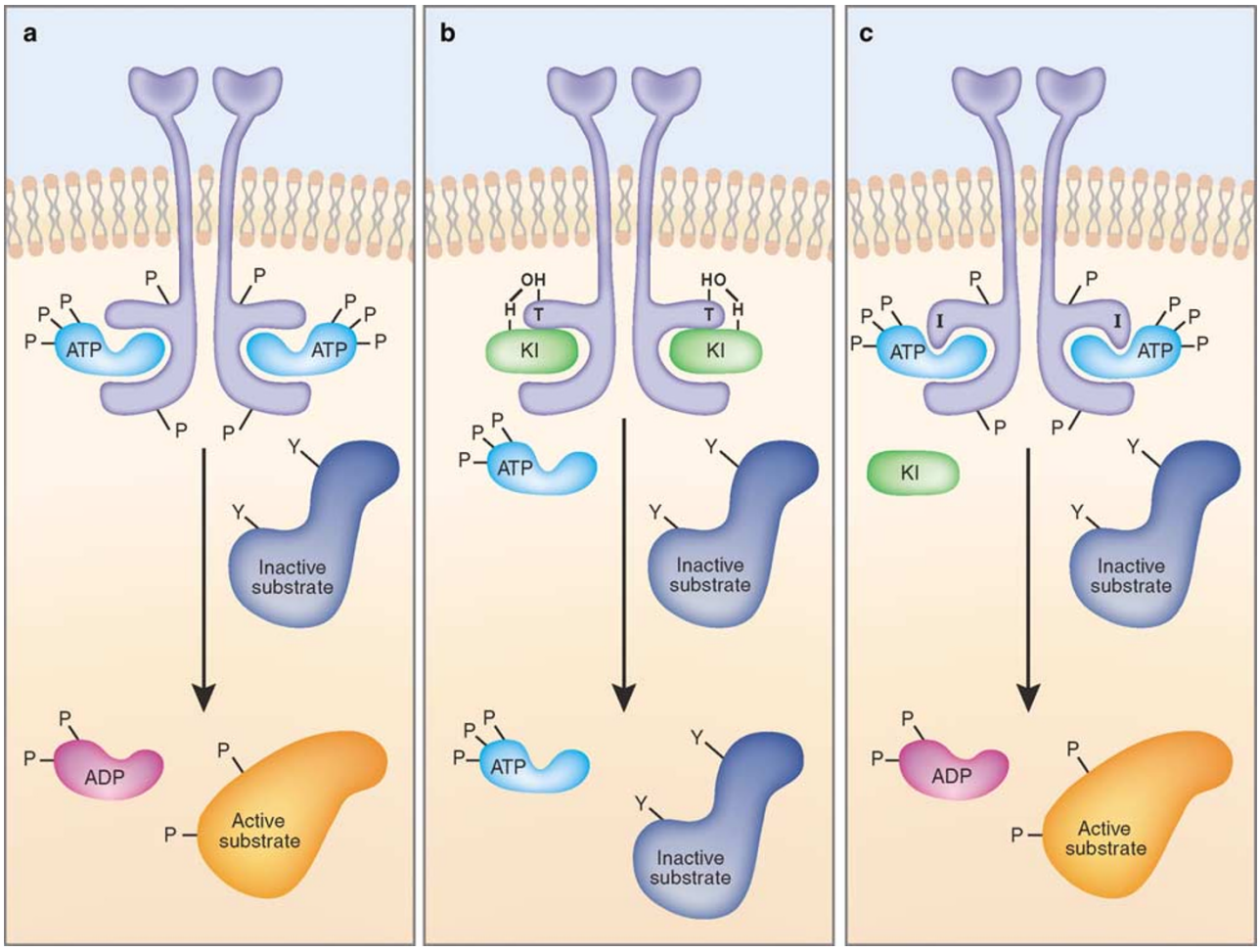

Figure 1 (**a**) Diagrammatic representation of oncogenically activated receptor tyrosine kinase, (**b**) inhibition of oncogenically activated kinase by small molecule inhibitor (KI), (**c**) resistance to small molecule inhibition secondary to a mutation that disrupts a critical hydrogen bond between the kinase inhibitor and the receptor tyrosine kinase and has a bulky side chain that interferes with binding ('gatekeeper mutation').

primary, targeted kinase, has been seen in GIST. Cases have been described that presented with a primary mutation in *KIT* exon 11 and acquired a secondary mutation in the kinase domain of *PDGFRA*,[12,28] a mutation that is also found in primary imatinib-resistant GISTs.[7,15] It is therefore likely that other, yet to be determined kinases including downstream effectors could also be activated in a similar manner in tumors targeted by kinase inhibitors.

Gene amplifications of *BCR-ABL* have been shown to contribute to secondary resistance in CML[25] and were recently described for *KIT* and *PDGFRA* in GISTs.[12,28] These presumably lead to higher expression levels, resulting in the need for higher doses of inhibitor to sufficiently inhibit the target. The situation is not as clear in NSCLC where *EGFR* gene amplifications can already be detected in tumors before onset of treatment.[29]

EGFR receptor internalization, however, has been experimentally shown to play a role in secondary resistance in NSCLC.[24] Drug-resistant cells showed altered receptor trafficking and demonstrated continued dependence on EGFR signaling without containing secondary *EGFR* mutations.

Additional mechanisms that can at least contribute to the resistant phenotype have been proposed, but most of them still need to be better defined. These include extracellular sequestration of the drug by plasma proteins as well as an enhanced active efflux by transmembrane pump proteins such as multidrug resistance (MDR)/p-glycoprotein.[25,30]

Strategies to overcome resistance

A number of strategies to overcome secondary drug resistance are currently being tested (summarized in Table 1). They can be divided into a search for compounds that target oncogenically activated kinases or drugs that inhibit downstream effectors. Interference with the activity of the oncogenic kinase can be accomplished through (i) more specific kinase inhibitors, (ii) multitargeted kinase

Table 1 Known mechanisms of resistance to kinase inhibition

Primary resistance	*Examples*
Mutations that inhibit binding of small molecule inhibitor	Some *PDGFRA* mutations in GIST[7,15]
Lack of mutations in target gene	*EGFR* in NSLC and *KIT* or *PDGFRA* in GIST[11]
Secondary resistance	*Examples*
Secondary mutations in target gene that abolish binding of inhibitor	Mutation of 'gatekeeper' residue: *BCR-ABL* (CML),[25] *KIT* (GIST)[12,17,20,21] and *EGFR* (NSCLC)[22–24] Mutations in the p-loop and activation loop: *BCR-ABL* (CML),[25] *KIT* (GIST)[12,18–21]
'Kinase Switch'	Mutation of *PDGFRA* in GISTs with primary *KIT* mutation[12,28] Upregulation of the LYN kinase in CML[44]
Amplification of gene target	*BCR-ABL* amplification in CML,[25] *KIT* and *PDGFRA* amplification in GIST[12,28]
Target receptor tyrosine kinase internalization	EGFR in NSCLC[24]
Extracellular sequestration of drug by plasma proteins	α1 acid glycoprotein for imatinib[25]
Drug efflux by transmembrane pump proteins	ABCB1 (multidrug resistance 1/p-glycoprotein 1) for imatinib[25] ABCG2 for gefitinib[30]

inhibitors, (iii) compounds that act differently from classical ATP-competitive binding, and (iv) compounds that reduce kinase expression or protein levels.

New kinase inhibitors have been developed that have an increased specificity for their target and the ability to inhibit kinases with resistance mutations. The rationale to design these more effective inhibitors is clear, especially in the case of imatinib. Compounds that bind to the oncogenic kinase in its active state (in contrast to imatinib) would be envisioned to be more efficient in target inhibition. New ABL-specific inhibitors have been developed that are 30- to 300-times more potent than imatinib and that bind to ABL in its active conformation.[25,31] Some of these compounds also inhibit KIT and PDGFRA and have entered clinical trials for GIST.[32,33]

A number of compounds are being developed that intentionally inhibit a broader spectrum of kinases (multitargeted inhibitors). The underlying principle is to reduce the formation of resistant cell clones by inhibiting two unrelated oncogenic mechanisms. For example, simultaneously targeting the desired oncogenic kinase plus vascular endothelial growth factor (VEGF) and PDGF receptors to inhibit the formation of new blood vessels is thought to increase the antitumor effects. Sunitinib malate (Sutent, SU11248, Pfizer, NY, USA), a multitargeted inhibitor that effectively blocks KIT, PDGF and VEGF receptors, RET and FLT3, was recently FDA-approved for the treatment of imatinib-resistant GIST (and metastatic renal cell carcinoma). It has also entered a phase II clinical trial for patients with advanced NSCLC.[31,34]

Several new compounds aim at inhibiting kinases by means other than competitively binding to the ATP-binding region. EGFR inhibitors that irreversibly bind to the receptor seem to be especially useful in NSCLC. A few compounds of this class have been shown to successfully inhibit the T790M resistance mutation, and some are in clinical trials.[22,24,35] A new group of allosteric inhibitors that impede the kinase by binding distantly to the active site have recently gained interest.[36] They putatively bind to the myristoyl pocket, exhibit exceptional target specificity and have synergistic antiproliferative effects when combined with imatinib.

A last group of compounds, characterized by heat shock protein (HSP) 90 inhibitors, does not target the phosphorylation mechanism of oncogenic kinases. HSPs are molecular chaperones that guide the normal folding, intracellular disposition and proteolytic turnover of many proteins.[37] Oncogenic mutations often lead to conformationally unstable proteins that need excessive amounts of chaperones to be stabilized. Consequently, inhibition of these chaperones should lead to increased proteasomal degradation of these mutant proteins.[37] HSP90 inhibitors have shown promising results in preclinical studies with GIST and NSCLC, and clinical trials have been initiated.[38,39]

A growing group of compounds that might help to overcome secondary resistance aims at the inhibition of signaling molecules downstream of oncogenically activated kinases. Important effectors of most tyrosine kinases are the PI3K/AKT/mTOR and RAS/RAF/MAPK pathways, respectively.[31,40] Examples in this category are inhibitors that target mTOR and are currently being tested in clinical trials for

GIST.[41] Moreover, inhibitors of PI3K and RAS are currently under investigation in gefitinib-resistant NSCLC.[42,43]

Finally, there are several possibilities of combination therapies that have in part found their way into clinical trials. These include regimens of the original compound plus conventional radio- or chemotherapy as well as treatments with the original drug plus the addition of one of the new compounds described above.

Summary

We have now entered the era of targeted therapy with small molecule inhibition of solid tumors. While primary resistance and secondary resistance will continue to be a problem, what we have learned so far has paved the way for further advances in this exciting area. The future looks bright for the treatment of solid tumors.

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Conflict of interest

The authors state no conflict of interest.

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