

## Perspectives in Pharmacology

# Role of Tyrosine Kinase Inhibitors in Cancer Therapy

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### ABSTRACT

Cancer chemotherapy has been one of the major medical advances in the last few decades. However, the drugs used for this therapy have a narrow therapeutic index, and often the responses produced are only just palliative as well as unpredictable. In contrast, targeted therapy that has been introduced in recent years is directed against cancer-specific molecules and signaling pathways and thus has more limited nonspecific toxicities. Tyrosine kinases are an especially important target because they play an important role in the modulation of growth factor signaling. This review focuses on small molecule inhibitors of tyrosine kinase. They compete with the ATP binding site of the catalytic domain of several oncogenic tyrosine kinases. They are orally active, small molecules that have a favorable safety profile and can be easily combined with other forms of chemotherapy or radiation therapy. Several tyrosine kinase inhibitors (TKIs) have been found to have effective antitumor

activity and have been approved or are in clinical trials. The inhibitors discussed in this manuscript are imatinib mesylate (STI571; Gleevec), gefitinib (Iressa), erlotinib (OSI-1774; Tarceva), lapatinib (GW-572016), canertinib (CI-1033), semaxinib (SU5416), vatalanib (PTK787/ZK222584), sorafenib (BAY 43-9006), sunitinib (SU11248), and leflunomide (SU101). TKIs are thus an important new class of targeted therapy that interfere with specific cell signaling pathways and thus allow target-specific therapy for selected malignancies. The pharmacological properties and anticancer activities of these inhibitors are discussed in this review. Use of these targeted therapies is not without limitations such as the development of resistance and the lack of tumor response in the general population. The availability of newer inhibitors and improved patient selection will help overcome these problems in the future.

Conventional chemotherapy, although directed toward certain macromolecules or enzymes, typically does not discriminate effectively between rapidly dividing normal cells (e.g., bone marrow and gastrointestinal tract) and tumor cells, thus leading to several toxic side effects. Tumor responses from cytotoxic chemotherapy are usually partial, brief, and unpredictable. In contrast, targeted therapies interfere with molecular targets that have a role in tumor growth or progression. These targets are usually located in tumor cells, although some like the antiangiogenic agents may target other cells such as endothelial cells. Thus, targeted therapies

have a high specificity toward tumor cells, providing a broader therapeutic window with less toxicity. They are also often useful in combination with cytotoxic chemotherapy or radiation to produce additive or synergistic anticancer activity because their toxicity profiles often do not overlap with traditional cytotoxic chemotherapy. Thus, targeted therapies represent a new and promising approach to cancer therapy, one that is already leading to beneficial clinical effects.

There are multiple types of targeted therapies available, including monoclonal antibodies, inhibitors of tyrosine kinases, and antisense inhibitors of growth factor receptors. This review focuses only on inhibitors of either receptor tyrosine kinases or nonreceptor tyrosine kinases. However, several other recent reviews focused on other types of tar-

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**ABBREVIATIONS:** EGFR, epidermal growth factor receptor(s); HER, human epidermal growth factor receptor; VEGFR, vascular endothelial growth factor receptor(s); PDGFR, platelet-derived growth factor receptor(s); Ph, Philadelphia chromosome; CML, chronic myeloid leukemia; ALL, acute lymphocytic leukemia; BMS-354825, *N*-(2-chloro-6-methylphenyl)-2-(6-(4-(2-hydroxyethyl)piperazin-1-yl)-2-methylpyrimidin-4-ylamino)thiazole-5-carboxamide; GIST, gastrointestinal stromal tumor; VEGF, vascular endothelial growth factor; NSCLC, nonsmall cell lung cancer; TRIBUTE, Tarceva Responses in Conjunction with Paclitaxel and Carboplatin; TALENT, Tarceva Lung Cancer Investigation; FLK, fetal liver kinase; KDR, kinase domain region; PDGF, platelet-derived growth factor; INTACT, Iressa NSCLC Trial Assessing Combination Treatment.

geted therapies such as monoclonal antibodies (Finley, 2003; Eskens, 2004). Tyrosine kinases play a critical role in the modulation of growth factor signaling. Activated forms of these enzymes can cause increases in tumor cell proliferation and growth, induce antiapoptotic effects, and promote angiogenesis and metastasis. In addition to activation by growth factors, protein kinase activation by somatic mutation is a common mechanism of tumor genesis. Because all of these effects are initiated by receptor tyrosine kinase activation, they are key targets for inhibitors. Table 1 illustrates some of the mutations associated with tumors and the tyrosine kinase inhibitors that act at those sites.

Tyrosine kinases are enzymes that catalyze the transfer of the  $\gamma$  phosphate group from adenosine triphosphate to target proteins. They play an important role in diverse normal cellular regulatory processes. Tyrosine kinases can be classified as receptor protein kinases and nonreceptor protein kinases. The receptor tyrosine kinases are membrane-spanning cell surface proteins that play critical roles in the transduction of extracellular signals to the cytoplasm (Pawson, 2002). There are approximately 60 receptor tyrosine kinases that have been identified, and they are divided into some 20 subfamilies as defined by receptor and/or ligand (Pawson, 2002). They are characterized by immunoglobulin-like sequences in their amino-terminal extracellular domains, a lipophilic transmembrane segment, and an intracellular carboxyl-terminal domain that includes the tyrosine kinase catalytic site (Heldin, 1995; Arteaga, 2001). Nonreceptor tyrosine kinases, on the other hand, relay intracellular signals.

Ligand binding induces dimerization of these receptor tyrosine kinases, resulting in autophosphorylation of their cytoplasmic domains and activation of tyrosine kinase activity. Multiple cytoplasmic signaling pathways, including the Ras/Raf mitogen-activated protein kinase pathway, the phosphoinositol 3'-kinase/Akt pathway, the signal transducer and activator of transcription 3 pathway, the protein kinase C pathway, and scaffolding proteins may then be activated (Schlessinger, 2000; Bogdan and Klambt, 2001). Intracellular mediators in these pathways transduce signals from membrane receptors through the cytosol and into the nucleus, culminating in altered DNA synthesis and cell division as well as effects on a variety of biological processes, including cell growth, migration, differentiation, and death (Carpenter and Cohen, 1990; Blume-Jensen and Hunter, 2001).

This review discusses the antitumor activity, mechanism of action, and adverse effects of several small molecule inhibitors of tyrosine kinases whose clinical effects have been fairly well defined (Fig. 1). These include imatinib, which inhibits the nonreceptor tyrosine kinases BCR-ABL and KIT, as well as receptor tyrosine kinase inhibitors targeting epi-

dermal growth factor receptor (EGFR) (ErbB/HER) family members, vascular endothelial growth factor receptors (VEGFR), and platelet-derived growth factor receptors (PDGFR) ( $\alpha$  and  $\beta$ ).

## BCR-ABL Tyrosine Kinase Inhibitors

### Imatinib Mesylate (STI571; Gleevec)

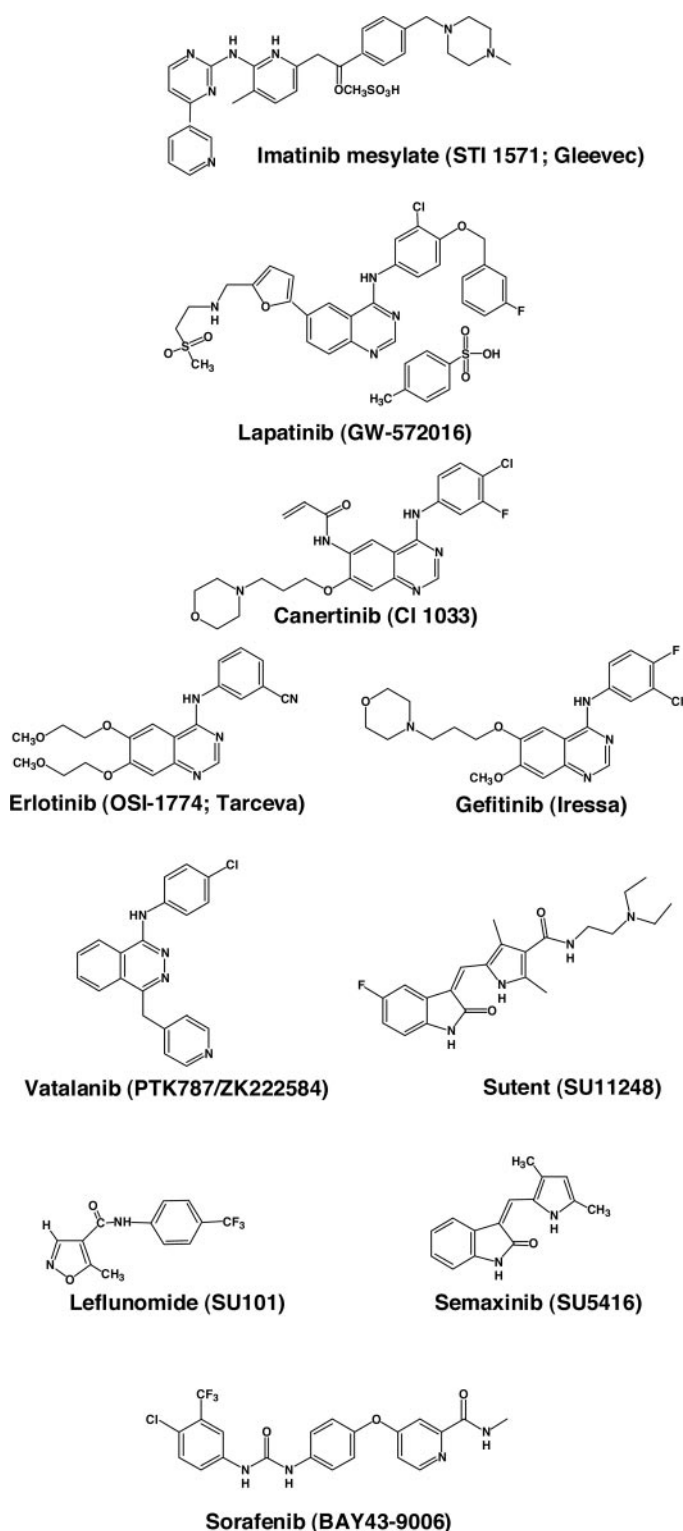
The t(9; 22) translocation or Philadelphia chromosome (Ph) is a characteristic cytogenetic abnormality seen in 95% of patients with chronic myeloid leukemia (CML) and 15 to 30% of adult patients with acute lymphoblastic leukemia (ALL) (Faderl et al., 1999; Shawver et al., 2002). This translocation results in the formation of the *BCR-ABL* oncogene by way of fusing the *BCR* gene on chromosome 22 and the *ABL* tyrosine kinase gene located on chromosome 9. This fusion results in the expression of two forms of protein-tyrosine kinases: p190 (BCR-ABL) and p210 (BCR-ABL). There is subsequent dysregulation of intracellular signaling with enhanced proliferative capability and resistance to apoptosis of hematopoietic stem or progenitor cells, which leads to a massive increase in myeloid cell numbers. The presence of this well defined pathogenetic defect at the molecular level led to the development of imatinib, which inhibits both the ABL and BCR-ABL tyrosine kinases (Druker et al., 1996).

The BCR-ABL protein is considered an ideal target for imatinib, since the BCR-ABL mutation is present in almost all patients with CML. Imatinib specifically inhibited or killed proliferating myeloid cell lines containing BCR-ABL but was minimally harmful to normal cells (Druker et al., 1996). Imatinib also reduced the formation of BCR-ABL-positive colonies by approximately 95% when cells from patients with CML were grown in colony-forming assays in vitro. It also suppressed the growth of Ph<sup>+</sup> ALL cells (Savage and Antman, 2002). The BCR-ABL protein is unique to leukemic cells and expressed at high levels, and its tyrosine kinase activity is essential for its ability to induce leukemia (Savage and Antman, 2002). Imatinib is used for the treatment of Ph<sup>+</sup> CML patients who are either newly diagnosed or have failed interferon- $\alpha$  therapy (Druker et al., 2001; Kantarjian et al., 2002a). Imatinib therapy induced major cytogenetic responses in 65 to 90% of patients with CML after failure to respond to interferon- $\alpha$  and in 80 to 90% of patients with previously untreated CML in the early chronic phase (Kantarjian et al., 2002b). Imatinib is also effective in the treatment of BCR/ABL-positive relapsed/refractory adult ALL, where 20 to 40% of the cases have this translocation. Complete responses were seen in 60 to 70% of cases, but most patients experienced relapse within months of treatment (Druker et al., 2001).

TABLE 1  
Representative mutations in cancer cells and inhibition by small molecule tyrosine kinase inhibitors

Target	Mutation	Tumor Type(s)	Tyrosine Kinase Inhibitor
BCR-ABL tyrosine kinase	<i>BCR-ABL</i> <sup>a</sup>	CML; ALL	Imatinib (STI 751; Gleevec)
c-KIT tyrosine kinase; PDGFR kinase	<i>c-KIT</i> <sup>b</sup>	GISTs	Imatinib (STI 751; Gleevec)
EGFR tyrosine kinase	<i>EGFR</i> tyrosine kinase domain <sup>c</sup>	Non-small cell lung cancer	Gefitinib (Iressa); Erlotinib (OSI-774; Tarceva)
FLT-3 kinase	<i>FLT-3-ITD</i> or <i>FLT-3D835</i> <sup>d</sup>	AML	Sutent (SU11248)
B-Raf kinase	<i>B-Raf V599E</i> <sup>e</sup>	Solid tumors (melanoma, renal carcinoma)	Sorafenib (BAY 43-9006)

<sup>a</sup> Druker (2004); <sup>b</sup> Savage and Antman (2002); <sup>c</sup> Pao and Miller (2005); <sup>d</sup> Gilliland and Griffin (2002); <sup>e</sup> Sharma et al. (2005).



**Fig. 1.** Structures of some representative small molecule tyrosine kinase inhibitors used in cancer therapy.

In some patients, white blood cells become resistant to imatinib, allowing the cancer to return; in addition, a significant number of newly diagnosed patients start out resistant. The most common resistance mechanism involves BCR-ABL kinase domain mutations that impart varying degrees of drug insensitivity (Gorre et al., 2001). Mutations at seventeen different amino acid positions within the BCR-ABL ki-

nase domain have been identified in imatinib resistance. Drug resistance is usually associated with the reactivation of BCR-ABL signal transduction, but *BCR-ABL* gene amplification and overexpression of protein is also associated with drug resistance both in vitro and in vivo (Gorre et al., 2001; Tsao et al., 2002). A new drug, BMS-354825, has now been recently developed by Bristol-Myers Squibb that binds to the active form of ABL and overcomes 14 of 15 imatinib-resistant mutants (Shah et al., 2004).

In addition to BCR-ABL, imatinib also inhibits the c-KIT and PDGFR tyrosine kinases. Dysregulation of c-KIT or PDGFR- $\alpha$  kinase is thought to play a role in gastrointestinal stromal tumor (GIST) formation (Hirota et al., 1998). These are rare tumors characterized by cell surface expression of the c-KIT, also known as CD117. Mutation of *c-KIT* leads to ligand-independent activation of the receptor. Imatinib inhibits the c-KIT tyrosine kinase at a concentration similar to the concentration required for the inhibition of BCR-ABL. Imatinib can block in vitro kinase activity of both wild-type KIT and a mutant KIT isoform commonly found in GISTs (Heinrich et al., 2000). Several clinical trials have shown a significant response to imatinib in patients with advanced GISTs (Druker, 2004). It is now approved for the treatment of patients with c-KIT-positive unresectable and/or malignant GISTs.

Imatinib therapy is generally well tolerated, and minimal side effects are observed compared with cytotoxic chemotherapy. Neutropenia, thrombocytopenia, and anemia occur in 35 to 45, 20, and 10% of patients, respectively, who are in the chronic phase of CML and receive standard-dose imatinib (Kantarjian et al., 2002a). Nonhematologic adverse effects include nausea, skin rash, peripheral edema, muscle cramps, and elevated liver transaminase levels (Kantarjian et al., 2002a). In patients treated for GISTs, myelosuppression was uncommon, although anemia did occur. Intratumoral and gastrointestinal bleeding developed in fewer than 5% of these patients (Savage and Antman, 2002).

## Epidermal Growth Factor Receptor Tyrosine Kinase Inhibitors

### Gefitinib (Iressa)

The EGFR family comprises four transmembrane tyrosine kinase growth factor receptors: EGFR itself (ErbB1) (EGFR/HER1), ErbB2 (HER2/neu), ErbB3 (HER3), and ErbB4 (HER4) (Ranson, 2004). Binding of a specific set of ligands to the receptor promotes EGFR dimerization and the autophosphorylation of the receptors on tyrosine residues (Arteaga, 2001). Upon autophosphorylation of the receptor, several signal transduction pathways downstream of EGFR become activated. The Ras/Raf mitogen-activated protein kinase pathway and the phosphoinositol 3'-kinase/Akt pathway are two major signaling routes for the HER family.

The EGFR signal transduction pathways have been implicated in the regulation of various neoplastic processes, including cell cycle progression, inhibition of apoptosis, tumor cell motility, invasion, and metastasis. EGFR activation also stimulates vascular endothelial growth factor (VEGF), which is the primary inducer of angiogenesis (Petit et al., 1997). In experimental models, deregulation of the EGFR-mediated signal transduction pathways is associated with oncogenesis (Wikstrand and Bigner, 1998). Mutations leading to contin-

uous activation or amplification and overexpression of EGFR proteins are seen in many human tumors, including tumors of the breast, lung, ovaries, and kidney. These mutations are a determinant of tumor aggressiveness (Wikstrand and Bigner, 1998). EGFR overexpression is frequently seen in non-small cell lung cancer (NSCLC), the most common cause of cancer-related death in the Western world (Dancey, 2004). The activity of EGFR can be inhibited either by blocking the extracellular ligand binding domain with the use of anti-EGFR antibodies or small molecules that inhibit the EGFR tyrosine kinase, thus resulting in inhibition of downstream components of the EGFR pathway (Mendelsohn, 1997).

Gefitinib is a selective EGFR (ErbB1) tyrosine kinase inhibitor. It has a 200-fold greater affinity for ErbB1 compared with that for ErbB2 (Thomas and Grandis, 2004). It prevents autophosphorylation of EGFR in various tumor cell lines and xenografts (Arteaga and Johnson, 2001). The specific mechanism of antitumor activity is not clear, but it is speculated that up-regulation of the cyclin-dependent kinase inhibitor p27 via EGFR kinase inhibition leads to inhibited cyclin-dependent kinase activity and arrest in the G<sub>1</sub> cell cycle phase (Arteaga and Johnson, 2001). Gefitinib can inhibit the growth of some ErbB2-overexpressing tumor cells (e.g., breast cancer) (Moulder et al., 2001; Normanno et al., 2002). It can also inhibit tumor neoangiogenesis (Arteaga and Johnson, 2001).

Gefitinib is approved for the treatment of patients with nonsmall cell lung cancer after failure of both platinum-based or docetaxel chemotherapies. Early results with gefitinib in lung cancer were encouraging, but results from large-scale randomized phase II trials were mixed. Approval was based on these studies of patients with refractory disease where the response rate was approximately 10% and the drug had a favorable safety profile (Fukuoka et al., 2003; Kris et al., 2003). Recently, point mutations in the EGFR tyrosine kinase domain in tumors from patients responding to EGFR kinase inhibitors were identified around the ATP-binding pocket of the tyrosine kinase domain of the *EGFR* gene. These provide a means of patient selection and perhaps a way of monitoring drug resistance (Dancey and Freidlin, 2003; Lynch et al., 2004). Most patients with nonsmall cell lung cancer have no response to gefitinib but in the subgroup with the mutations the response rate was approximately 10% (Lynch et al., 2004).

Adverse effects seen after gefitinib administration were generally mild and resolved after discontinuation of the drug. The most common adverse effects were diarrhea, rash, acne, dry skin, nausea, vomiting, pruritus, anorexia, and asthenia. All EGFR-targeting agents cause an acneiform rash that is thought to reflect EGFR inhibition in the skin (Dancey and Freidlin, 2003). Adverse effects occasionally reported included fatigue, serum transaminase elevations, stomatitis, bone pain, dyspnea, and pulmonary toxicity. The possibility of pulmonary toxicity is one of the most controversial issues surrounding gefitinib administration. The main concern is the possibility of interstitial lung disease, also referred to as alveolitis, pneumonitis, and interstitial pneumonia (Cersosimo, 2004). The highest rate of this was in Japan. A safety warning was issued in the fall of 2001, and by early 2003, Japanese officials associated over 170 deaths with gefitinib-related interstitial lung disease.

### Erlotinib (OSI-774; Tarceva)

Erlotinib hydrochloride is an orally available, potent, reversible, and selective inhibitor of the EGFR (ErbB1) tyrosine kinase (Moyer et al., 1997; Ranson, 2004). Studies in human cancer cells found that it inhibits epidermal growth factor-dependent cell proliferation at nanomolar concentrations and blocks cell cycle progression in the G<sub>1</sub> phase (Moyer et al., 1997).

Erlotinib was approved by the Food and Drug Administration in November 2004. In a placebo-controlled trial, patients randomized to erlotinib with advanced stage III or IV NSCLC and who had progressive disease after standard chemotherapies showed improved symptoms and increased survival. The response rate was 12%, and the median survival was 8.4 months (Perez-Soler, 2004). In another trial with stage IIIB or IV advanced or recurrent metastatic NSCLC after platinum-based therapy and in patients who were positive for ErbB1, erlotinib therapy was associated with tumor-related symptom improvement (Herbst, 2003). In a phase II study in patients with pure bronchoalveolar carcinoma or adenocarcinoma of lung with bronchoalveolar carcinoma features, cigarette smoking predicted sensitivity to erlotinib. Patients who never smoked or smoked with less than or equal to one pack/year for 5 years or its equivalent had higher response rates (Kris et al., 2004). However, two randomized phase III studies (TRIBUTE and TALENT) used erlotinib with carboplatin/paclitaxel or cisplatin/gemcitabine. The addition of erlotinib did not produce a survival advantage over chemotherapy alone (Herbst et al., 2004). Erlotinib, when combined with trastuzumab in patients with ErbB2-positive metastatic breast cancer in a phase I trial, showed that this combination provided a well tolerated targeted therapy with preliminary evidence of antitumor activity (Britten et al., 2004). Erlotinib is also under investigation in several other tumor types, including pancreatic and colon cancer in combination with chemotherapy (Herbst, 2003; Schiller, 2003; Thomas and Grandis, 2004).

The most frequent adverse effects seen with erlotinib are an acneiform skin rash and diarrhea. Diarrhea is the dose-limiting adverse event. Headache, mucositis, hyperbilirubinemia, neutropenia, and anemia have also been reported (Ranson et al., 2002; Ranson, 2004).

### Lapatinib (GW-572016)

Lapatinib is a reversible and specific receptor tyrosine kinase inhibitor of both ErbB1 and ErbB2 and has been shown to have activity against ErbB1 and ErbB2, as well as Akt-overexpressing human tumor xenografts (Rusnak et al., 2001). Its nonselective inhibition of EGFR may account for a broader spectrum of antitumor activity and improved efficacy. It may also be possible that the development of resistance is less likely. Lapatinib also inhibits baseline p95ErbB2 (truncated ErbB2 receptor) phosphorylation in vitro and in tumor xenografts (Xia et al., 2004). Phase I data have been reported with notable tumor responses seen in patients with trastuzumab refractory breast cancer and in nonsmall cell lung cancer (Spector et al., 2003). A phase II study with metastatic colorectal cancer is in progress (Belanger et al., 2003). The most common adverse effects associated with the use of lapatinib were diarrhea and skin rash.

### Canertinib (CI-1033)

Canertinib is an irreversible nonselective EGFR inhibitor. This characteristic may result in a greater efficacy and broader spectrum of antitumor activity. Irreversible inhibitors may also have the advantage of prolonged clinical effects and a need for less frequent dosing; however, it may compromise specificity and tolerability. Canertinib produces rapid, irreversible inhibition of all members of the EGFR family (Ranson, 2004). It inhibits EGFR kinase activity with an  $IC_{50}$  in the low nanomolar range and has antitumor activity in ErbB1- and ErbB2-dependent preclinical models (Slichenmyer et al., 2001). It is also active against ErbB3 and B4 but has no effect on other tyrosine kinases (Thomas and Grandis, 2004).

Canertinib has been shown to have activity against a variety of human breast carcinomas in both *in vitro* and *in vivo* tumor xenograft models (Allen et al., 2002). Phase II studies in progressive or recurrent locally advanced or metastatic nonsmall cell lung cancer and metastatic breast cancers are ongoing (<http://www.clinicaltrials.gov/>). In one phase II study in platinum refractory ovarian cancer patients, canertinib has shown minimal activity, i.e., stable disease with no objective responses (Campos et al., 2004). The most common adverse events associated with this agent were diarrhea, nausea, skin rash, vomiting, asthenia, and stomatitis (Rowinski et al., 2003).

### Vascular Endothelial Growth Factor Tyrosine Kinase Inhibitors

Angiogenesis is a complex process that occurs in a variety of physiologic and pathophysiologic states and is a remodeling of an established primitive network of blood vessels (Keyhani et al., 2001; Alessi et al., 2004). VEGF is secreted by all almost all solid tumors and tumor-associated stroma in response to hypoxia. It is highly specific for vascular endothelium and regulates both vascular proliferation and permeability. Excessive expression of VEGF levels correlate with increased microvascular density, cancer recurrence, and decreased survival (Parikh and Ellis, 2004).

There are six different ligands for the VEGFR, VEGF-A through -E and placenta growth factor. Ligands bind to specific receptors on endothelial cells, mostly VEGFR-2 (FLK-1/KDR), but it will also bind to VEGFR-1 (Flt-1) and -3. The binding of VEGF-A to VEGFR-1 induces endothelial cell migration. VEGFR-2 induces endothelial cell proliferation, permeability, and survival. VEGFR-3 is thought to mediate lymphangiogenesis. Binding of VEGF to VEGFR-2 receptors results in activation and autophosphorylation of intracellular tyrosine kinase domains, with triggering of intracellular signaling cascade (Bergsland, 2004; Parikh and Ellis, 2004).

### Semaxinib (SU5416)

Semaxinib is a small, lipophilic, highly protein-bound nonselective receptor tyrosine kinase inhibitor of VEGFR-2, c-KIT, and FLT-3 (Mendel et al., 2000). This compound showed antiangiogenic and antitumor activity in preclinical studies and was the first VEGFR tyrosine kinase inhibitor to be tested clinically (Stopeck et al., 2003). In a multicenter phase II study with twice weekly semaxinib, one complete and seven partial responses were observed in patients with refractory acute myeloid leukemia or in elderly patients med-

ically unfit for intensive induction chemotherapy (Fiedler et al., 2003). Randomized phase III studies of semaxinib with 5-fluorouracil/leucovorin and 5-fluorouracil/leucovorin/irinotecan in patients with metastatic colorectal carcinoma failed to show a survival benefit of the semaxinib-containing regimens (Eskens, 2004). No objective response rates were seen in phase II studies with prostate cancer, renal cell cancer, and multiple myeloma. Toxicities of semaxinib include headache, nausea, vomiting, asthenia, pain at the infusion site, phlebitis, change in voice, and fevers. Semaxinib has to be dissolved in a cremophor plus ethanol vehicle, thus requiring coadministration with steroids to prevent hypersensitivity reactions.

### Vatalanib (PTK787/ZK222584)

Vatalanib is a potent, orally active, selective inhibitor of the VEGFR tyrosine kinases VEGFR-1 (Flt-1) and VEGFR-2 (FLK-1/KDR). It is most potent against VEGFR-2 and exhibits slightly weaker inhibition of VEGFR-1. At higher concentrations, it also inhibits other tyrosine kinases, including PDGFR- $\beta$ , c-KIT, and c-FMS (Lin et al., 2002). In contrast, it is not active against the EGFR, fibroblast growth factor receptor-1, c-MET, and TIE-2 or intracellular kinases such as c-SRC, c-ABL, and protein kinase C- $\alpha$  (Lin et al., 2002; Rini and Small, 2005).

Vatalanib reduces growth and the microvasculature in subcutaneously implanted human tumor xenografts in rodent models. It was also shown to reduce vessel density in tumor tissues without a direct effect on any of these tumor cells, suggesting that its primary mode of action in these cells is through inhibition of angiogenesis (Lin et al., 2002; Rini and Small, 2005). Studies have shown that vatalanib can directly act on multiple myeloma cells and in the bone marrow milieu to inhibit multiple myeloma cell growth and survival and overcome drug resistance (Lin et al., 2002). These VEGF-mediated responses can be effectively blocked with vatalanib.

Vatalanib is being studied as a single agent and in combination with chemotherapy in patients with colorectal cancer and liver metastases, advanced prostate and renal cell cancer, and relapsed/refractory glioblastoma multiforme, where VEGF overexpression has been demonstrated (Steward et al., 2003; Bergsland, 2004). In the renal carcinoma studies, partial responses were seen in 5% of patients, and minor responses were seen in 15% of patients (Rini and Small, 2005). Ataxia, vertigo, and hypertension are dose-limiting toxicities. Some incidences of venous thromboembolism also occurred (Eskens, 2004).

### Sutent (SU11248)

Sutent is a broad-spectrum, orally available multitargeted tyrosine kinase inhibitor of VEGFR, PDGFR, c-KIT, and FLT-3 kinase activity (Mendel et al., 2002). It inhibits the growth of a variety of mouse tumor cells and xenograft models (Bergsland, 2004; Traxler et al., 2004). Phase I trials have noted tumor regressions and antiangiogenic activity, and phase II studies in patients with metastatic kidney cancer found that 33% of patients had a partial response and 37% had stable disease for longer than 3 months on the therapy (Eskens, 2004; Motzer et al., 2004). Phase III clinical trials with kidney cancer using sutent as a single agent and in combination chemotherapy are ongoing. It has demonstrated

both efficacy and safety in these trials. It is also being studied in a phase III trial for imatinib-resistant GISTs. Sutent delayed the time of tumor progression on average from 1.5 to 6.3 months and also significantly reduced the death rate (Demetri et al., 2005).

### Sorafenib (BAY 43-9006)

Sorafenib is a novel dual-action Raf kinase and VEGFR inhibitor that inhibits tumor cell proliferation and angiogenesis. Although originally developed as a Raf kinase inhibitor, it was subsequently found to inhibit a variety of kinase receptors, including VEGFR, EGFR, and PDGFR kinases (Wilhelm et al., 2004; Strumberg et al., 2005). A specific Raf kinase, B-Raf, is mutated in two-thirds of melanomas and a small percentage of colorectal and other solid tumors (Davies et al., 2002). This leads to elevated Raf kinase activity and cellular proliferation. Sorafenib had significant activity in four different tumor types, including renal, colon, pancreatic, lung, and ovarian tumors (Wilhelm et al., 2004).

A phase II randomized clinical trial in patients with advanced kidney cancer found that after a 12-week treatment period there were a statistically higher percentage of patients whose disease did not progress in the BAY 43-9006 group compared with the placebo. In addition, 70% of the patients with tumors had tumor shrinkage or disease stabilization (Ratain et al., 2004). Furthermore, it was reported to produce partial responses in a phase I/II clinical study when administered in combination with carboplatin and paclitaxel in patients with advanced malignant melanoma (Ahmad et al., 2004). Phase III studies are in progress. The most commonly reported adverse effects were skin reactions such as hand-foot syndrome and rash, diarrhea, fatigue, weight loss, and hypertension, all of which were manageable and reversible.

## Platelet-Derived Growth Factor Inhibitors

### Leflunomide (SU101)

Platelet-derived growth factor (PDGF) signals through a cell surface tyrosine kinase receptor (PDGFR) to stimulate various cellular functions, including growth, proliferation, and differentiation (Sedlacek, 2000). Two distinct PDGFR types have been identified:  $\alpha$  and  $\beta$ . Intracellular activation of this receptor can lead to cell transformation and generation of a mitotic signal. Both receptor types are overexpressed in several solid tumors as well as in the surrounding stroma (Sedlacek, 2000).

Leflunomide is a small molecule inhibitor of PDGFR-mediated phosphorylation and thus inhibits PDGF-mediated cell signaling (Shawver et al., 1997). Leflunomide is converted to its principal metabolite, SU0020, which interferes with de novo pyrimidine synthesis. At this time, it is not clear whether the mechanism of action of this drug in humans is due to inhibition of PDGF-dependent signaling, inhibition of pyrimidine synthesis, or a combination of both (Ko et al., 2001; Adamson et al., 2004; Olsen and Stein, 2004). Leflunomide is an immunomodulatory agent that is indicated in adults for treatment of active rheumatoid arthritis. It reduces signs and symptoms of the disease and retards structural damage. Extensive preclinical data also suggests a role for immunosuppression with leflunomide in organ transplantation. It has also demonstrated broad-spectrum antitumor

activity in preclinical studies. Studies with tumor xenografts have shown that leflunomide induced greater growth inhibition in xenografts that expressed PDGFR compared with xenografts not expressing this receptor. A multi-institutional phase II study in hormone refractory prostate cancer patients with leflunomide found partial responses in 1 of 19 patients, a prostate-specific antigen decline greater than 50% in 3 of 39 patients, and improvement in pain (Ko et al., 2001). A phase II/III randomized trial has now completed accrual for comparing the effectiveness of mitoxanthone and prednisone with or without leflunomide in patients with stage IV prostate cancer that have not responded to hormone therapy (<http://www.clinicaltrials.gov/>). The most frequently reported side effects with leflunomide were asthenia, nausea, anorexia, and anemia.

## Summary

Targeted therapy refers to a new generation of anticancer drugs that are designed to interfere with a specific molecular target, usually a protein with a critical role in tumor growth or progression. This approach differs from the more empirical approach used in conventional cytotoxic chemotherapy, which has remained the mainstay of anticancer drug use over the past several decades (Sawyers, 2004). Targeted therapy has the potential to reduce or eliminate many of the present problems in the field of cytotoxic chemotherapy, such as the production of serious host-cell toxicity. Several types of targeted therapy are available, but this review focuses in particular only on small molecule tyrosine kinase inhibitors. Three have been approved for use in cancer therapy, and several others are in various stages of clinical trials. Table 2 provides a summary of these agents and their use in cancer therapy.

Imatinib, one of the first and most effective small molecule tyrosine kinase inhibitors, serves as a model for the development of other tyrosine kinase inhibitors and for targeted therapy in general (Druker, 2004). Its targets, BCR-ABL tyrosine kinase and CML, have several features that are critical to the success of this agent and are likely to predict the success of other targeted therapies. The BCR-ABL tyrosine kinase has clearly been shown to be critical to the pathogenesis of CML. Imatinib is highly selective for this mutated kinase. As with most malignancies, the best results are obtained when therapy for CML is begun early. Thus, identification of crucial early events in malignant progression is a key step in replicating the success of imatinib with other tumors. Another important issue is selecting patients for clinical trials on the basis of an appropriate target. With CML, activation of *BCR-ABL* was easily identifiable by the presence of the Philadelphia chromosome. Together, these factors account for the excellent results obtained. Similarly, in GISTs, the main cause is mutations in the c-KIT tyrosine kinase. Imatinib also inhibits the activity of the c-KIT and PDGF- $\alpha$  kinases and produces dramatic clinical responses in GIST similar to those in CML (Pardnani and Tefferi, 2004).

If key features of imatinib can be replicated with other inhibitors, targeted therapy holds great promise; however, there are limitations and drawbacks to this type of therapy. One of these is disease relapse due to drug resistance. The best understanding of this problem at a molecular level comes from studies of imatinib resistance in CML patients.

TABLE 2  
Anticancer activity of tyrosine kinase inhibitors

Inhibitor	Tyrosine Kinase Target	IC <sub>50</sub> for Kinase Inhibition	Neoplasm(s) Targeted	Clinical Status
Imatinib mesylate (STI 751; Gleevec)	BCR-ABL	<i>nM</i> 100 <sup>a,b</sup>	CML; GISTs	Approved
	c-KIT	100 <sup>a</sup>		
	PDGFR	100 <sup>a</sup>		
Gefitinib (Iressa)	ErbB1 (EGFR)	33 <sup>b</sup>	Lung cancer	Approved
Erlotinib (OSI-774; Tarceva)	ErbB1 (EGFR)	2 <sup>c</sup>	Lung cancer	Approved
Lapatinib (GW-572016)	ErbB1	10 <sup>d</sup>	Breast cancer; other solid tumors	Phase II
	ErbB2	98 <sup>d</sup>		
Canertinib (CI-1033)	EGFR (nonselective)	17 ErbB1 <sup>c</sup> 9 ErbB2 <sup>c</sup>	Breast cancer	Phase I/II
Semaxinib (SU5416)	VEGFR-2	1040 <sup>e</sup>	AML	Phase I/II
Vatalanib (PTK787/ZK222584)	c-KIT	77 <sup>f</sup>	Colorectal cancer; prostate and renal cancer	Phase I/II
	FLT-3			
	VEGFR-1 (Flt-1)			
Sutent (SU11248)	VEGF-2 (FLK-1/KDR)	37 <sup>f</sup>	GIST; renal cancer	Phase II/III
	VEGFR	10 <sup>g</sup>		
	PDGFR	10 <sup>g</sup>		
	c-KIT			
Sorafenib (BAY 43-9006)	FLT-3	38 <sup>h</sup> 90 <sup>h</sup>	Renal cancer; malignant melanoma	Phase II/III
	B-Raf			
	VEGFR-2			
Leflunomide (SU101)	PDGFR	65 <sup>i</sup>	Prostate cancer	Phase II/III

<sup>a</sup> Lyseng-Williamson and Jarvis (2001); <sup>b</sup> Tibes et al. (2005); <sup>c</sup> Thomas and Grandis (2004); <sup>d</sup> Rusnak et al. (2001); <sup>e</sup> Fong et al. (1999); <sup>f</sup> Lin et al. (2002); <sup>g</sup> Mendel et al. (2003); <sup>h</sup> Wilhelm et al. (2004); <sup>i</sup> Shawver et al. (1997).

Relapse results from an expansion of resistant tumor subclones in the face of continued therapy. These subclones contain amino acid mutations in the BCR-ABL kinase domain that prevent enzyme inhibition by imatinib (Gorre et al., 2001). Fortunately, second-generation kinase inhibitors are now available that retain activity against nearly all of the imatinib-resistant mutants (Shah et al., 2004). These compounds are now in early clinical testing. Future therapies are likely to rely on combinations of inhibitors to prevent the emergence of resistance.

Another potential limitation to targeted therapy is the possibility of multiple mutations. The *BCR-ABL* mutation in CML and the *c-KIT* mutations in GISTs are exceptions rather than the rule in that most cancers do not have a single mutation present in almost all patients. For targeted therapies to be successful, they will have to be designed to work against cancers with multiple lesions.

Successful clinical use of targeted therapy also hinges on the ability to recognize the molecular phenotype of tumors likely to respond to therapy and to monitor target inhibition in the tumors during treatment. Specific target inhibition could also be used to guide dose selection and interpret clinical results. This contributed to the success of imatinib for the BCR-ABL kinase, but an urgent need for biomarker studies is seen in the recent experience with the EGFR kinase inhibitors gefitinib and erlotinib. Although 10 to 20% of patients treated with gefitinib seemed to respond to therapy in early clinical trials, subsequent larger-scale studies failed to reveal how to select those patients most likely to respond. Fortunately, the recent identification of specific point mutations in the *EGFR* gene in tumors from patients responding to gefitinib and their absence in nonresponders now provides a mechanism for selecting patients. Monitoring for maintenance of these mutations may also be useful in understanding drug resistance (Lynch et al., 2004).

Poor patient selection may also have accounted for the disappointing results achieved with erlotinib and gefitinib in

the INTACT-1, INTACT-2, TRIBUTE, and TALENT trials. In these trials, combinations with cytotoxic chemotherapy showed no significant benefits. In addition to poor patient selection, other reasons for these results may have been antagonistic effects between the kinase inhibitors and the cytotoxic agents. Furthermore, in the clinical trials, the inhibitor was given continuously without interruptions, whereas in the preclinical studies the animals did not receive the EGFR inhibitor until 48 h prior to chemotherapy and might have been sensitized to the effects of the cytotoxic agents due to release of the tumor cells from G<sub>1</sub>-S arrest (Perez-Soler, 2004).

For the antiangiogenic agents (e.g., semaxinib, etc.), there may be additional explanations for the poor responses obtained in cancers to date. VEGF is thought to be the most potent direct-acting stimulatory regulator of angiogenesis, and expression of VEGF is excessive in human cancers; however, there are a myriad of stimulatory and inhibitory factors involved in angiogenesis. Some of these are produced by tumor cells, and some are produced by host cells. In addition, for each angiogenic factor, there are multiple regulatory factors and multiple signaling pathways that exist. With all this redundancy, inhibiting one factor or one pathway often will not be sufficient to inhibit tumor growth. Furthermore, some of the factors such as VEGF exist in multiple isoforms, adding to the difficulty of inhibiting the angiogenic process (Parikh and Ellis, 2004).

Targeted therapy provides a new approach for cancer therapy that has the potential for avoiding some of the drawbacks associated with cytotoxic chemotherapy. Unfortunately, several of the present-generation small molecule tyrosine kinase inhibitors used in targeted therapy have their drawbacks and limitations and have more similarities than differences to the current cytotoxic drugs. However, knowledge of their effects will facilitate the development of improved targeted agents that can circumvent these limitations.

At the present time, tyrosine kinase inhibitors serve more

as second- or third-line therapies rather than as primary therapy. They may also be useful in combination with traditional cytotoxic chemotherapy. For the tyrosine kinase inhibitors to have a primary role in therapy, there has to be a clear hypothesis for their use, relevant preclinical data, and a demonstrated use in well characterized groups of patients. So far, these criteria have not been met for most of the presently available tyrosine kinase inhibitors.

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