

Role of Tyrosine Kinase Inhibitors in Cancer Therapy

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Abbreviations: tyrosine kinase inhibitors, (TKIs); receptor tyrosine kinases, (RTKIs); non-receptor tyrosine kinases (NRTKs); vascular endothelial growth factor (VEGF);chronic myeloid leukemia (CML); Philadelphia chromosome (Ph);acute lymphocytic leukemia, (ALL);gastrointestinal stromal tumor (GIST);epidermal growth factor receptor (EGFR);non-small cell lung cancer (NSCLC); platelet derived growth factor (PDGF); platelet derived growth factor receptor, (PDGFR); fibroblast growth factor (FGF);fibroblast growth factor receptor (FGFR)

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. Targeted therapy which has been introduced in recent years, in contrast, is directed against cancer-specific molecules and signaling pathways and thus has more limited non-specific toxicities. Tyrosine kinases are an especially important target as they play an important role in the modulation of growth factor signaling. This manuscript 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 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.

Introduction

Conventional chemotherapy, although directed towards certain macromolecules or enzymes, typically does not discriminate effectively between rapidly dividing normal cells (e.g. bone marrow, GI 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 (RTKs) or non-receptor tyrosine kinases (nRTKs). However, several other recent reviews focus on other types of targeted therapies such as monoclonal antibodies (Eskens, 2004; Finley, 2003). 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 which act at those sites.

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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 non-receptor 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 RTKs 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 (Arteaga, 2001; Heldin, 1995). 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 PI3K (phosphoinositol 3'-kinase)/Akt pathway, the signal transducer and activator of transcription 3 (STAT3) pathway, the protein kinase C (PKC) pathway and scaffolding proteins may then be activated (Bogdan and Klambt, 2001; Schlessinger, 2000). 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. These include imatinib, which inhibits the non-receptor tyrosine kinases BCR-ABL and KIT, as well as receptor tyrosine kinase inhibitors targeting EGFR (ERB B/HER) family members,

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vascular endothelial growth factor (VEGF) receptors, platelet derived growth factor receptors (PDGF receptors α and β).

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 15-30% of adult patients with acute lymphoblastic leukemia (ALL) (Faderl et al., 1999; Shawver et al., 2002). This translocation results in 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 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 about 95% when cells from patients with CML were grown in colony-forming assays *in vitro*. It also suppressed the growth of Ph⁺ 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⁺ CML patients who are either newly diagnosed or have failed interferon-alpha therapy (Kantarjian et al. 2002, Druker et al., 2001). Imatinib therapy induced major cytogenetic responses in 65-90%

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of patients with CML after failure to respond to interferon α and in 80-90% of patients with previously untreated CML in the early chronic phase (Kantarjian et al. 2002a). 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 60-70% of cases, but most patients experienced relapse within months of treatment (Druker et al., 2001).

In some patients, white blood cells become resistant to imatinib allowing the cancer to return and 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 kinase 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 over expression 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 α 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.

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

Epidermal growth factor receptor tyrosine kinase inhibitors

Gefitinib (Iressa)

The epidermal growth factor receptor (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 receptors autophosphorylation on tyrosine residues (Arteaga, 2001). Upon autophosphorylation of the receptor several signal transduction pathways downstream of EGF-R become activated. The Ras-Raf MAP kinase pathway and the PI3K -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 continuous activation or amplification and over expression of EGFR proteins are seen in many human tumors, including tumors of the breast,

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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). Activity of EGFR can be inhibited either by blocking the extracellular ligand binding domain with the use of anti-EGFR antibodies or by the use of 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 to 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 (CDK)inhibitor p27 via EGFR kinase inhibition leads to inhibited CDK activity and arrest in the G1 cell-cycle phase (Arteaga and Johnson, 2001). Gefitinib can inhibit the growth of some ErbB2 over expressing tumor cells (eg, 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 non-small 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 about 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 (Lynch et al., 2004; Dancey and Freidlin, 2003). Most patients with non-small cell lung cancer have no response

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to gefitinib but in the subgroup with the mutations the response rate was about 10% (Lynch et al., 2004)

Adverse effects seen after gefitinib administration were in general 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 which 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 (Ranson, 2004; Moyer et al., JD, 1997). 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 G₁ phase (Moyer et al., 1997).

Erlotinib was approved by the FDA 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 (BAC) or adenocarcinoma of lung with BAC features, cigarette smoking predicted sensitivity to

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erlotinib. Patients who never smoked or who in patients with less than 5 pack years had higher response rates (Kris et al., 2004). However, two randomized phase III studies (TRIBUTE) and (TALENT) used erlotinib with carboplatin/paclitaxel or with 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 anti-tumor 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, ErbB2 as well as AKT overexpressing human tumors xenografts (Rusnak et al., 2001). Its non selective inhibition of EGF receptors may account for a broader spectrum of antitumor activity and improved efficacy. It may also 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 non-small 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)

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Canertinib is an irreversible nonselective EGFR inhibitor. This characteristic may result in a greater efficacy and a 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 non-small cell lung cancer and metastatic breast cancers are ongoing (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, 2004). The most common adverse events associated with this agent were diarrhea, nausea, skin rash, vomiting, asthenia and stomatitis (Rowinski, 2003).

Vascular endothelial growth factor tyrosine kinase inhibitors

Angiogenesis is a complex process that occurs in a variety of physiologic and patho-physiologic states and is a remodeling of an established primitive network of blood vessels (Alessi et al., 2004). Vascular Endothelial Growth Factor (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).

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There are 6 different ligands for the VEGF receptor, VEGF-A through -E and placenta growth factor (PlGF). 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 (Parikh and Ellis, 2004; Bergsland, 2004).

Semaxinib (SU5416)

Semaxinib is a small, lipophilic, highly protein-bound non-selective receptor tyrosine kinase inhibitor (RTKI) of VEGFR-2, c-KIT and FLT3 (Mendel et al., 2000). This compound showed antiangiogenic and antitumor activity in preclinical studies and was the first VEGF RTKI 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 medically unfit for intensive induction chemotherapy (Fiedler et al., 2003). Randomized phase III studies of semaxinib with 5-FU/leucovorin and 5-FU/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/ZK 222584)

Vatalanib is a potent, orally active and selective inhibitor of the VEGF-receptor 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

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tyrosine kinases including the platelet derived growth factor (PDGF) receptor β , c-KIT, and c-FMS (Lin et al, 2002). In contrast, it is not active against the EGFR, fibroblast growth factor (FGF) receptor-1, c-MET, and TIE-2, or intracellular kinases such as c-SRC, c-ABL and protein kinase C- α (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 (Rini and Small, 2005; Lin et al., 2002). 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; George et al., 2001; Bergsland, 2004). In the renal carcinoma studies partial responses were seen in 5% of patients and minor responses 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 multi-targeted 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 33% of patients has 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

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with kidney cancer with sunitinib 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. Sunitinib 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 (Bignell 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 as compared to the placebo. Also 70% of the patients with tumors had tumor shrinkage or disease stabilization (Ratain et al., 2004). In addition, 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 (PDGF) Inhibitors

Leflunomide (SU 101)

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

Leflunomide is a small molecule inhibitor of PDGF receptor-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 if the mechanism of action of this drug in humans is due to inhibition of PDGF-dependent signaling, or inhibition of pyrimidine synthesis or a combination of both (Olsen and Stein, 2004; Ko et al., 2001; Adamson et al., 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 anti-tumor activity in preclinical studies. Studies with tumor xenografts have shown that leflunomide induced greater growth inhibition in xenografts that expressed PDGFR when compared to xenografts not expressing this receptor. A multi-institutional phase II study in hormone refractory prostate cancer patients with leflunomide found partial responses in 1/19 patients, a prostate specific antigen (PSA) decline greater than 50% in 3/39 patients and improvement in pain (Ko et al., 2001). A phase II/III randomized trial has now completed accrual for comparing the effectiveness of mitoxantrone and prednisone with or without leflunomide in patients with stage IV prostate cancer that has not responded to hormone therapy (www.clinicaltrials.gov)

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trials.gov). The most frequently reported side effects with leflunomide were asthenia, nausea, anorexia and anemia.

Summary

Targeted therapy refers to a new generation of cancer 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 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. Two of them 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 target, BCR-ABL tyrosine kinase and chronic myelogenous leukemia 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 imatinib's success 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

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also inhibits the activity of the c-KIT and PDGF- α kinases and produces dramatic clinical responses in GIST similar to those in CML (Pardanani 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. 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 10-20 % of patients treated with gefitinib appeared 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

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the *EGFR* gene in tumors from patients responding to gefitinib and their absence in non-responders 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, and TRIBUTE and TALENT trials. In these trials combinations with cytotoxic chemotherapy showed no significant benefits. Besides poor patient selection other reasons for these results may have been antagonistic effects between the kinase inhibitors and the cytotoxic agents. Also in the clinical trials the inhibitor was given continuously without interruptions while in the preclinical studies the animals did not receive the EGFR inhibitor until 48 hours prior to chemotherapy and might have been sensitized to the effects of the cytotoxic agents due to release of the tumor cells from G1-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 by host cells. Also 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. In addition, some of the factors like 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

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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 TKIs 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 TKIs.

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FIGURE LEGEND

Figure 1: Structures of some representative small molecule tyrosine kinase inhibitors used in cancer therapy.

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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> ^a	CML, ALL	Imatinib (STI 751; Gleevec)
c-KIT tyrosine kinase, PDGFR kinase	<i>c-KIT</i> ^b	Gastrointestinal stromal tumors (GISTs)	Imatinib (STI 751; Gleevec)
EGFR tyrosine kinase/	<i>EGFR</i> tyrosine kinase domain ^c	Non-small cell lung cancer	Gefitinib (Iressa), Erlotinib (OSI 774; Tarceva)
FLT3 kinase	<i>FLT3-ITD</i> or <i>FLT3D835</i> ^d	AML	Sutent (SU11248)
B-RAF kinase	<i>B-RAF V599E</i> ^e	Solid tumors (melanoma, renal carcinoma)	Sorafenib (BAY 43-9006)

^afrom Druker, 2004; ^bfrom Savage and Antman, 2002; ^cfrom Pao and Miller, 2005; ^dfrom Gilliland and Griffin, 2002 ^efrom Sharma et al., 2005

TABLE 2
Anticancer activity of Tyrosine Kinase Inhibitors

Inhibitor	Tyrosine kinase target	IC ₅₀ (nM) for kinase inhibition	Neoplasm (s) targeted	Clinical status
Imatinib mesylate (STI 751;Gleevec)	BCR-ABL c-KIT PDGFR	100 ^{a,b} 100 ^a 100 ^a	CML, GISTs	Approved
Gefitinib (Iressa)	ErbB1 (EGFR)	33 ^b	Lung cancer	Approved
Erlotinib (OSI 774;Tarceva)	ErbB1 (EGFR)	2 ^c	Lung cancer	Approved
Lapatinib (GW572016)	ErbB1 Erb B2	10 ^d 98 ^d	Breast cancer; other solid tumors	Phase II
Canertinib (CI-1033)	EGFR (nonselective)	17 ErbB1 ^c ; 9 Erb B2 ^c	Breast cancer	Phase I/II
Semaxinib (SU 5416)	VEGFR-2 c-KIT FLT3	1040 ^e - -	AML	Phase I/II
Vatalanib (PTK 787/ZK222584)	VEGFR-1(Flt-1) VEGF-2 (FLK-1/KDR)	77 ^f 37 ^f	Colorectal cancer, prostate and renal cancer	Phase I/II
Sutent (SU11248)	VEGFR PDGFR KIT FLT3	10 ^g 10 ^g - -	GIST, renal cancer,	Phase II/III
Sorafenib (BAY 43-9006)	B-RAF VEGFR-2	38 ^h 90 ^h	Renal cancer, malignant melanoma	Phase II/III
Leflunomide (SU101)	PDGFR	65 ⁱ	Prostate cancer	Phase II/III

^afrom Lyseng-Williams and Jarvis, 2001 ; ^bfrom Tibes et al., 2005; ^cfrom Thomas and Grandis, 2004; ^dfrom Rusnak et al., 2001; ^efrom Fong et al., 1999; ^ffrom Lin et al., 2002; ^gMendel et al., 2003; ^hfrom Wilhelm et al., 2004; and ⁱfrom Shawver et al., 1997

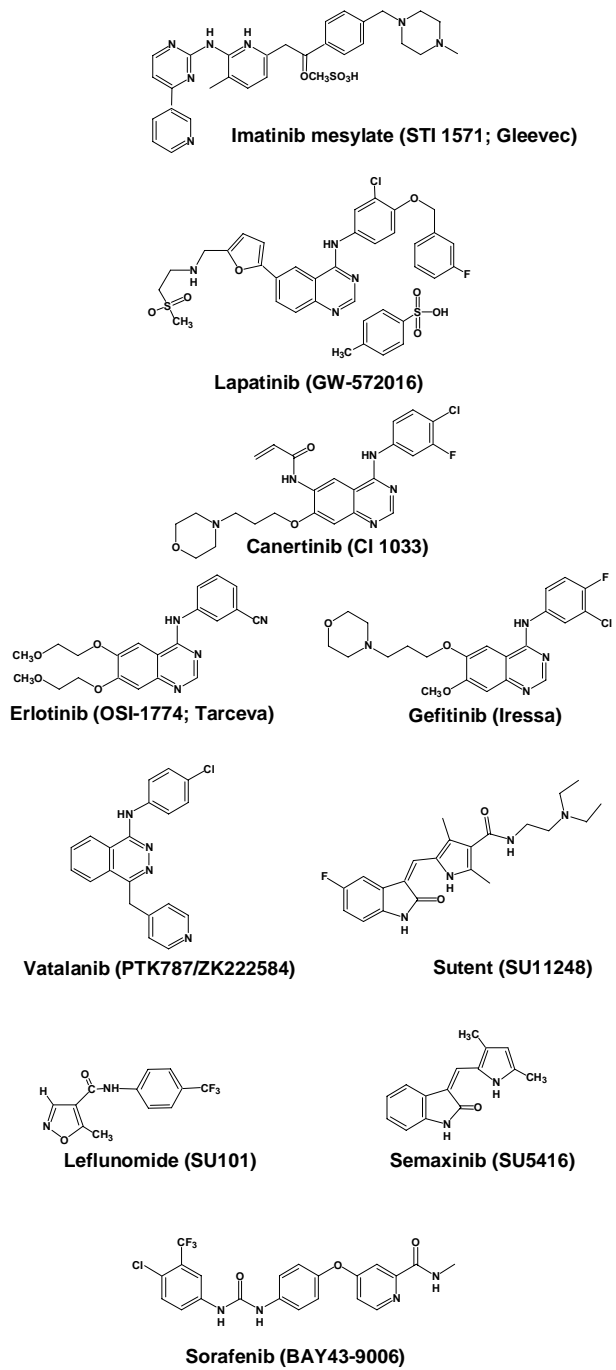


Fig. 1.