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Tyrosine Kinases: promising targets for cancer chemotherapy

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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), sutent (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.

Keywords: Kinase; Protein kinase; Kinase inhibitors; Cancer therapy

Introduction

Kinase is a type of enzyme that transfers phosphate groups from high-energy donor molecules, such as ATPs to specific substrates, a process referred to as phosphorylation. Kinases are part of the larger family of phosphotransferases. Kinases are not to be confused with

phosphorylases, which carry out phosphorolysis, the breaking of a bond using an inorganic phosphate group; or with phosphatases, which remove phosphate groups. (Hunter 2000; Schlessinger 2000).

Types

One of the largest groups of kinases are protein kinases, which act on and modify the activity of specific proteins. Kinases are used extensively to transmit signals and control complex processes in cells. More than five hundred different kinases have been identified in humans. Their enormous diversity, as well as their role in signaling, makes them an object of study.Various other kinases act on small molecules such as lipids, carbohydrates, amino acids, and nucleotides, either for signaling or to prime them for metabolic pathways. Kinases are often named after their substrates. (Blume-Jensen 2001)

Types of protein kinase

serine/threonine protein kinase

serine/threonine protein kinase is a kinase enzyme that phosphorylates the OH group of serine or threonine (which has similar side chains). At least 125 of the 500 plus human protein kinases are serine/threonine kinases (STK).

Casein kinase

The Casein kinase is a serine/threonine-selective protein kinase that is a tetramer of two alpha subunits and two beta subunits. The alpha subunits have the catalytic kinase domain. Casein kinase has been implicated in cell cycle control, DNA repair, regulation of the circadian rhythm and other cellular processes. Casein kinase activity has been reported to be activated following Wnt signaling pathway activation. A Pertussis toxin-sensitive G protein and Disheveled appear to be an intermediary between Wnt-mediated activation of the Frizzled receptor and activation of casein kinase. Mice that lack casein kinase 2 alpha prime have a defect in the morphology of developing sperm. (Carpenter 1975; Hunter 1978).

Protein kinase A

In cell biology, Protein kinase A (PKA) refers to a family of enzymes whose activity is dependent on cellular levels of cyclic AMP (cAMP). PKA is also known as cAMP-dependent protein kinase. (Workman 2003) Protein kinase A has several functions in the cell, including regulation of glycogen, sugar, and lipid metabolism. It should neither be confused with AMP-activated protein kinase - which, although being of similar nature, may have opposite effects nor with cyclin-dependent kinases (Cdks).

Protein kinase C

Protein kinase C also known as PKC is a family of protein kinase enzymes that are involved in controlling the function of other proteins through the phosphorylation of hydroxyl groups of serine and threonine amino acid residues on these proteins. PKC enzymes in

turn are activated by signals such as increases in the concentration of diacylglycerol (DAG) or calcium ions (Ca^{2+}). Hence PKC enzymes play important roles in several signal transduction cascades. The PKC family consists of fifteen isozymes in humans. They are divided into three subfamilies, based on their second messenger requirements: conventional (or classical), novel, and atypical. Conventional (c) PKCs contains the isoforms α, β_I, β_{II}, and γ. These require Ca^{2+}, DAG, and a phospholipid such as phosphatidylserine for activation. Novel (n) PKCs include the δ, ε, η, and θ isoforms, and require DAG, but do not require Ca^{2+} for activation. Thus, conventional and novel PKCs are activated through the same signal transduction pathway as phospholipase C. On the other hand, atypical (a) PKCs (including protein kinase M ζ and ι / λ isoforms) require neither Ca^{2+} nor diacylglycerol for activation. The term "protein kinase C" usually refers to the entire family of isoforms. (Hunter 1995; Sawyers 2002).

Tyrosine kinases are important mediators of this signal transduction process, leading to cell proliferation, differentiation, migration, metabolism and programmed cell death. Tyrosine kinases are a family of enzymes, which catalyzes phosphorylation of select tyrosine residues in target proteins, using ATP. This covalent post-translational modification is a pivotal component of normal cellular communication and maintenance of homeostasis. (Schenk 1999) Tyrosine kinases are implicated in several steps of neoplastic development and progression. Tyrosine kinase signaling pathways normally prevent deregulated proliferat-ion or contribute to sensitivity towards apoptotic stimuli. These signaling pathways are often genetically or epigenetically altered in cancer cells to impart a selection advantage to the cancer cells. Thus, it is no wonder that aberrant enhanced signaling emanating from tyrosine kinase endows these enzymes a dominating oncoprotein status, resulting in the malfunctio-ning of signaling network. (Heldin 1995).

The discovery that SRC oncogen having a transforming non receptor tyrosine kinase activity (Rowley 2012) , and the finding of EGFR, the first receptor tyrosine kinase paved the way to the understanding of the role and significance of tyrosine kinase in cancer. (Normile 2012) With the deciphering of the Human Genome Project more than 90 tyrosine kinases have been found out. The more science entangles the intricacies of cellular signaling the more we find the involvement of tyrosine kinase in cellular signaling circuits that are implicated in cancer development. Tyrosine kinases represent a major portion of all oncoprotein that play a transforming role in a plethora of cancers. Hence the identification and development of therapeutic agents for disease states that are linked to abnormal activation of tyrosine kinases due to enhanced expression, mutation or autocrine stimulation leading to abnormal downstream oncogenic signaling have taken a centre stage as a potent target for cancer therapy (Druker 2009).

Kinases regulate cell function by transferring a phosphate group from ATP to recipient proteins, thereby influencing the activity, localization and overall function of the d-ownstream substrate proteins. Phosphorylation occurs mainly at serine and threonine but also at tyrosine residues. Tyrosine kinases are often part of a cell surface receptor complex and are auto phoshorylated upon binding of a ligand to the extracellular part of the receptor. (Gambacorti-Passerini, 2008). Serine-threonine kinases are regulators of the intracellular signaling networks. More than 500 kinases are encoded in the human genome, making kinases one of the largest families of related genes, with many kinases still uncharacterized (Druker et al ,

2000) Kinases are involved in virtually all physiological activities in the human body and have been identified as important therapeutic targets. (Kluin-Nelemans et al , 2006) Most of the pharmaceutical firms have kinase inhibitors underdevelopment with furthest progress for kinase inhibitor start getting pathways.

Biochemical mechanism of tyrosine kinases

Tyrosine kinases are enzymes that selectively phosphorylates tyrosine residue in different substrates. Receptor tyrosine kinases are activated by ligand binding to their extracellular domain.Ligands are extracellular signal molecules (e.g. EGF, PDGF etc) that induce receptor dimerization(except Insulin receptor). Different ligands employ different strategies by which they achieve the stable dimeric conformation. One ligand may bind with two receptor molecules to form 1:2 ligand: receptor complex e.g. growth hormone and growth hormone receptor, while in other cases two ligands binds simultaneously to two receptors 2:2 ligand receptor complex and provides the simplest mechanism of receptor dimerization e.g. VEGF and VEGFR. The receptor dimerization is also stabilized by receptor–receptor interactions. Some ligand receptor is not sufficient for some complex and is stabilized by accessory molecules e.g. FGFs are unable to activate FGFR complex and is stabilized by heparin sulfate proteoglycans (HSPG). Ligand binding to the extracellular domain stabilizes the formation of active dimmers and consequently protein tyrosine kinase activation. Structural studies of the catalytic core of several RTKs, supported by biochemical and kinetic studies of receptor phosphorylation have provided proof that receptor oligomerization increases the local concentration of the RTKs, leading to efficient transphosphorylation of tyrosine residues in the activation loop of the catalytic domain. Upon tyrosine phosphorylation the activation loop adopts an open conformation that gives access to ATP and substrates and makes ATP transfer from Mg-ATP to tyrosine residue on the receptor itself and on cellular proteins involved in signal transduction. ATP binding intracellular catalytic domain that catalyzes receptor auto phosphorylation displays the highest level of conservation between the RTKs. The ATP binding site serves as a docking site for specific binding of cytoplasmic signaling proteins containing Src homology-2 (SH2) and protein tyrosine binding (PTB) domains. These proteins in turn recruit additional effector molecules having SH2, SH3, PTB and Pleckstrin homology (PH) domain. This results in the assembly of signaling complexes to the activated receptor and the membrane and subsequent activation of a cascade of intracellular biochemical signals, which leads to the activation or repression of various subsets of genes and thus defines the biological response to signals. During these processes, receptors migrate within the plasma membrane and are internalized through clathrin-coated invagination, which eventually seal off and forms an endocytic vesicle. The endocytic vesicles fuse with the lysosomes and in the process the receptor and ligand may be degraded by the lysosomal enzymes. The receptors are also recycled in some cases. During the whole process of receptor internalization the ligand receptor complex is dissociated and this results in the termination of the signaling reaction.

Classification

Tyrosine kinases are primarily classified as receptor tyrosine kinase (RTK) e.g. EGFR, PDGFR, FGFR and the IR and non-receptor tyrosine kinase (NRTK) e.g. SRC, ABL, FAK and Janus kinase. The receptor tyrosine kinases are not only cell surface trans membra-

ne receptors, but are also enzymes having kinase activity. The structural organization of the receptor tyrosine kinase exhibits a multi domain extracellular ligand for conveying ligand specificity, a single pass trans membrane hydrophobic helix and a cytoplasmic portion containing a tyrosine kinase domain. The kinase domain has regulatory sequence both on the N and C terminal end. (Yang 2008) NRTK are cytoplasmic proteins, exhibiting considerable structural variability. The NRTK have a kinase domain and often possess several additional signaling or protein-protein interacting domains such as SH2, SH3 and the PH domain. The tyrosine kinase domain spans approximately 300residues and consists of an N terminal lobe comprising of a 5 stranded β sheet and one α helix, while the C terminal domain is a large cytoplasmic domain that is mainly α helical. ATP binds in the cleft in between the two lobes and the tyrosine containing sequence of the protein substrate interacts with the sides of the C terminal lobe. RTK are activated by ligand binding to the extracellular domain followed by dimerization of receptors, facilitating trans-phosphorylation in the cytoplasmic domain whereas the activation mechanism of NRTK is more complex, involving heterologous protein-protein interaction to enable transphosphorylation.

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. (Tapper et al , 2009) 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. There is subsequent dys-regulation 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. (Boucher et al, 2003). 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 (Fig 1) specifically inhibited or killed proliferating myeloid cell lines containing BCR-ABL but was minimally harmful to normal cells. 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^+ ALL cells. (Lassila et al, 2004) The BCR-ABL protein is unique to leukemic cells and expressed

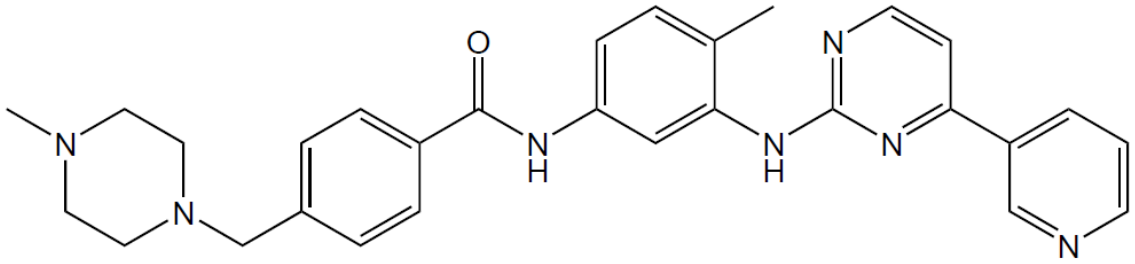

Figure 1. Imatinib

at high levels, and its tyrosine kinase activity is essential for its ability to induce leukemia. Imatinib is used for the treatment of Ph^+ CML patients who are either newly diagnosed or have failed interferon-α therapy. (Reeves et al , 2005) Imatinib therapy induced major cytogenetic responses in 65 to 90% of patients with CML after failure to respond to interferonα and in 80 to 90% of patients with previously untreated CML in the early chronic phase. 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.

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. (Li P. et al 2006) 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. 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. (Holmes et al, 2008)

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 gastrointest-inal stromal tumor (GIST) formation. (Haberfeld, 2008) 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 isoforms commonly found in GISTs. Several clinical trials have shown a significant response to imatinib in patients with advanced GISTs. 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. Nonhematologic adverse effects include nausea, skin rash, peripheral edema, muscle cramps, and elevated liver transaminase levels. In patients treated for GISTs, myelosuppression was uncommon, although anemia did occur. Intratumoral and gastrointestinal bleeding developed in fewer than 5% of these patients. (Kerkelä R. et al , 2006)

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). Binding of a specific set of ligands to the receptor promotes EGFR dimeriza-

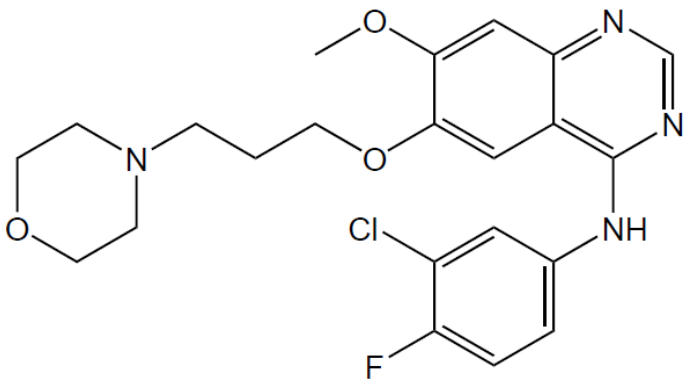

Figure 2. Gefitinib (Iressa)

tion and the autophosphorylation of the receptors on tyrosine residues. 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. 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. Gefitinib (Fig 2) is a selective EGFR (ErbB1) tyrosine kinase inhibitor. It has a 200-fold greater affinity for ErbB1 compared with that for ErbB2. It prevents autophosphorylation of EGFR in various tumor cell lines and xenografts. 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_1 cell cycle phase. Gefitinib can inhibit the growth of some ErbB2-overexpressing tumor cells (e.g., breast cancer) 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 encoura-ging, 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 approxim-ately 10% and the drug had a favorable safety profile. 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. Most patients with nonsmall cell lung cancer have no response to gefitinib but in the subgr-oup with the mutations the response rate was approximately 10%. (Shima H et al , 2011)

Erlotinib (OSI-774; Tarceva)

Erlotinib hydrochloride is an orally available, potent, reversible, and selective inhibitor of the EGFR (ErbB1) tyrosine kinase. Studies in human cancer cells found that it inhibits

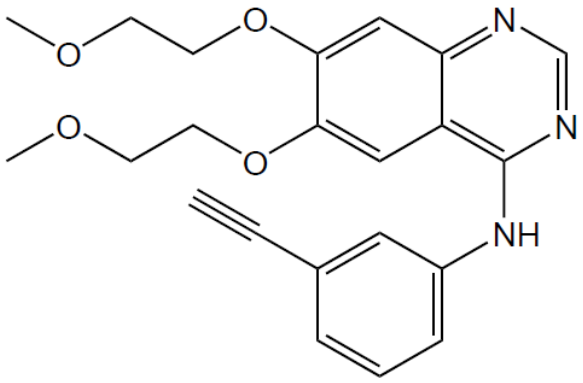

Figure 3. Erlotinib (OSI-774; Tarceva)

epidermal growth factor-dependent cell proliferation at nanomolar concentrations and blocks cell cycle progression in the G_1 phase. Erlotinib (Fig 3) 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 chem-otherapies showed improved symptoms and increased survival. The response rate was 12%, and the median survival was 8.4 months. (Scheinfel, 2006) 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. 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. However, two randomized phase III studies erlotinib along with carboplatin/paclitaxel or cisplatin/gemcitabine. The addition of erlotinib did not produce a survival advantage over chemotherapy alone. Erlotin-ib, 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 wi-th preliminary evidence of antitumor activity. Erlotinib is also under investigation in several other tumor types, including pancreatic and colon cancer in combination with chemotherapy. (Takimoto , 2008)

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. 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. (Gunby et al, 2003) 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. A phase II study with metastatic colorectal cancer is in progress. The most common adverse effects associated with the use of lapatinib (Fig 4) were diarrhea and skin rash.

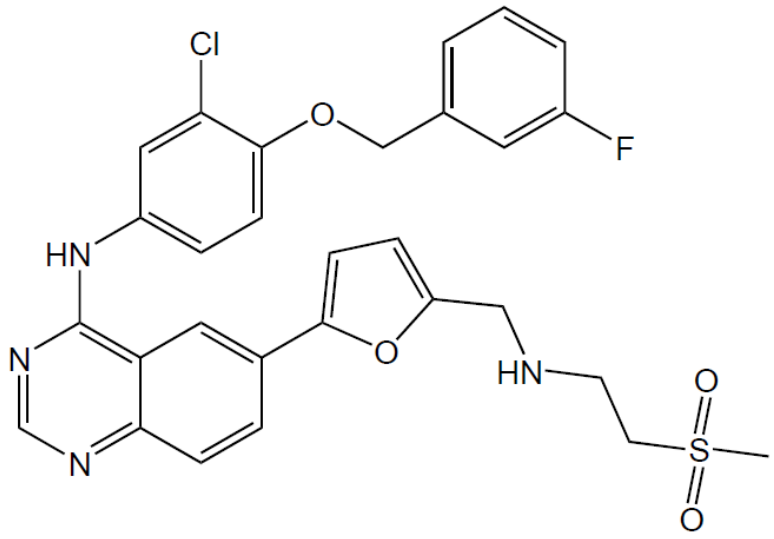

Figure 4. Lapatinib (GW-572016)

Canertinib (CI-1033)

Canertinib (Fig 5) is an irreversible nonselective EGFR inhibitor. This characteristic may res-ult 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. 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. It is also active against ErbB3 and B4 but has no effect on other tyrosine kinases. (Deininger , 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 ves-

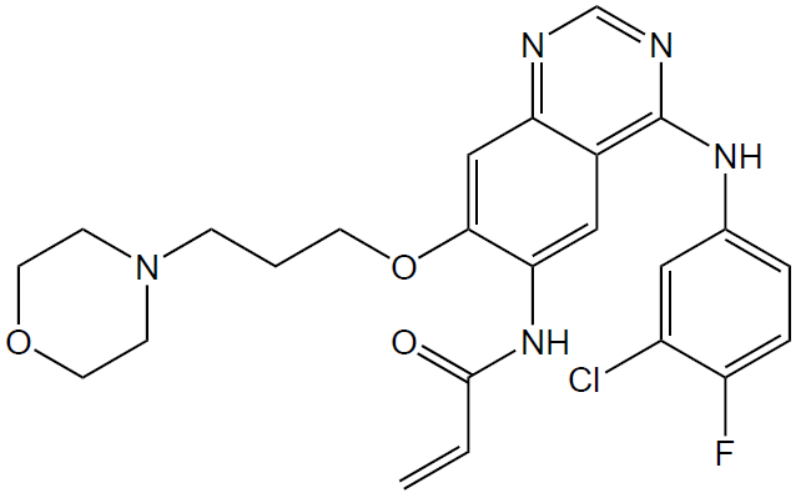

Figure 5. Canertinib (CI-1033)

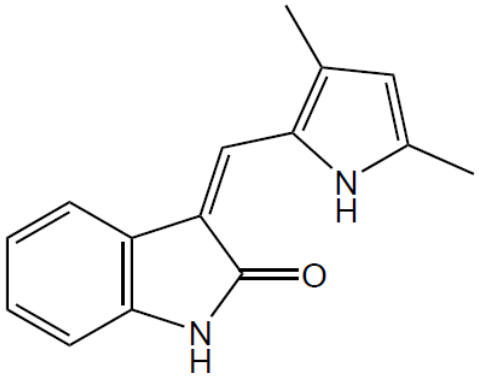

Figure 6. Semaxinib (SU5416)

sels. 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. 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.

Semaxinib (SU5416)

Semaxinib (Fig. 6) is a small, lipophilic, highly protein-bound nonselective receptor tyrosine kinase inhibitor of VEGFR-2, c-KIT, and FLT-3. This compound showed antiangiogenic and antitumor activity in preclinical studies and was the first VEGFR tyrosine kinase. inhibitor to be tested clinically. 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. 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. 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. (Vigneri, 2001).

Vatalanib (PTK787/ZK222584)

Vatalanib (Fig. 7) 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

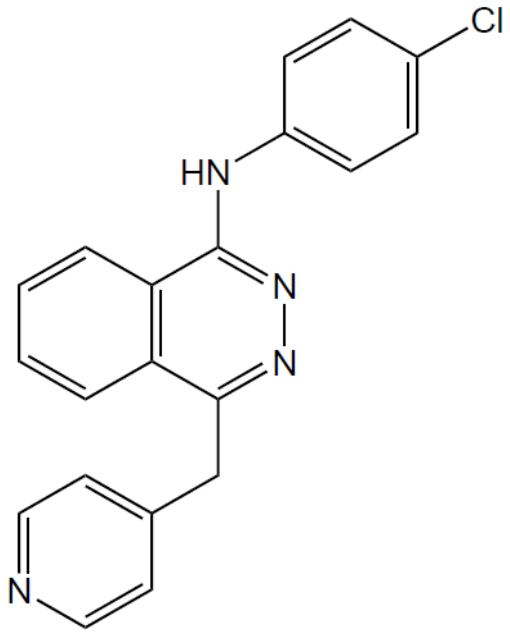

Figure 7. Vatalanib (PTK787/ZK222584

and exhibits slightly weaker inhibition of VEGFR-1. At higher concentrations, it also inhibits other tyrosine kinases, including PDGFR-β, c-KIT, and c-FMS. 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-α. 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 inhibit-ion of angiogenesis. 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. 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 over expression has been demonstrated. In the renal carcinoma studies, partial responses were seen in 5% of patients, and minor responses were seen in 15% of patients. Ataxia, vertigo, and hypertension are dose-limiting toxicities. Some incidences of venous thrombo embolism also occurred.

Sunitinib (Sutent, SU11248)

Sunitinib (marketed as Sutent. Fig 8) is a broad-spectrum, orally available multi-targeted tyrosine kinase inhibitor of VEGFR, PDGFR, c-KIT, and FLT-3 kinase activi-ty. It inhibits the growth of a variety of mouse tumor cells and xenograft models. 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. Phase III clinical trials with kidney cancer using sutent as a single agent and in combination chemotherapy are ongoing. It has

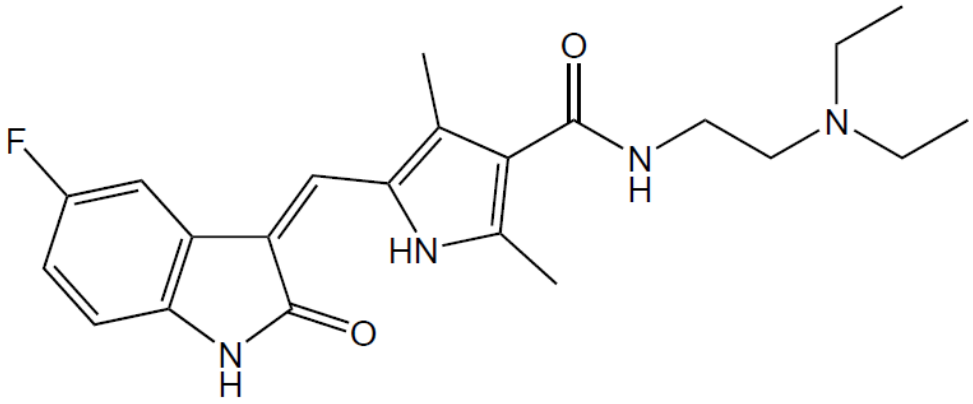

Figure 8. Sunitinib (Sutent, SU11248)

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.

Sorafenib (BAY 43-9006)

Sorafenib (Fig. 9) 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. A specific Raf kinase, B-Raf, is mutated in two-thirds of melanomas and a small percentage of colorectal and other solid tumors. 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. 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. 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. 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.(Klopp, 2010; Manley et al 2007)

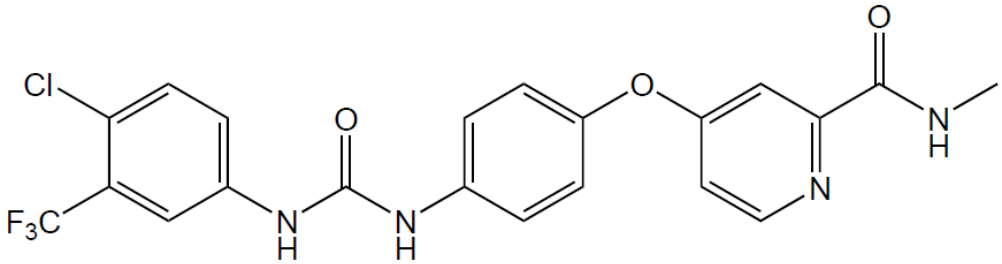

Figure 9. Sorafenib (BAY 43-9006)

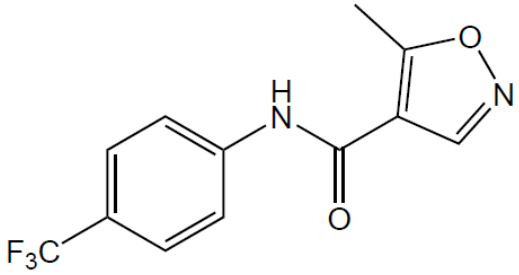

Figure 10. Leflunomide (SU101)

Platelet-Derived Growth Factor Inhibitors

Platelet-derived growth factor (PDGF) signals through a cell surface tyrosine kinase receptor (PDGFR) to stimulate various cellular functions, including growth, proliferation, and differentiation. Two distinct PDGFR 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. (Schiffer 2007)

Leflunomide (SU101)

Leflunomide (Fig.10) is a small molecule inhibitor of PDGFR-mediated phosphorylation and thus inhibits PDGF-mediated cell signaling. 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. Leflunomide is an immunomodulatory agent that is indicated in adults for treatment of active rheumatoid arthritis.(Pollack 2009) 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.(Brody 2010) 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. 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. The most frequently reported side effects with leflunomide were asthenia, nausea, anorexia, and anemia. (Kelley et al, 2010)

Conclusion

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 grow-

th 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. 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. 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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