Annu. Rev. Pharmacol. Toxicol. 2005. 45:357–84
doi: 10.1146/annurev.pharmtox.45.120403.100124

First published online as a Review in Advance on September 27, 2004

TYROSINE KINASE INHIBITORS AND THE DAWN OF MOLECULAR CANCER THERAPEUTICS*

Raoul Tibes, Jonathan Trent, and Razelle Kurzrock
Division of Cancer Medicine, University of Texas M.D. Anderson Cancer Center, Houston, Texas 77030; email: rkurzroc@mdanderson.org

Key Words imatinib mesylate, Bcr-Abl, KIT, kinase, cancer

■ **Abstract** The clinical application of tyrosine kinase inhibitors for cancer treatment represents a therapeutic breakthrough. The rationale for developing these compounds rests on the observation that tyrosine kinase enzymes are critical components of the cellular signaling apparatus and are regularly mutated or otherwise deregulated in human malignancies. Novel tyrosine kinase inhibitors are designed to exploit the molecular differences between tumor cells and normal tissues. Herein, we will review the current state-of-the-art using agents that target as prototypes Bcr-Abl, platelet-derived growth factor receptor (PDGFR), *KIT* (stem cell factor receptor), and epidermal growth factor receptor (EGFR). These compounds are remarkably effective in treating diverse cancers that are highly resistant to conventional treatment, including various forms of leukemia, hypereosinophilic syndrome, mast cell disease, sarcomas, and lung cancer. It is now clear that the molecular defects underlying cancer can be targeted with designer drugs that yield striking salutary effects with minimal toxicity.

INTRODUCTION

Cancer is the second most common cause of death in developed countries and is a rising health problem in less developed parts of the world. The diagnosis of cancer carries great physical and mental suffering for affected individuals and poses a significant burden on the health care system. For many tumors, conventional management strategies (surgery, radiation, chemotherapy) have high toxicity with

*Abbreviations: ATF2: Activating transcription factor; ERK: Extracellular regulated kinase; GCK: Glucokinase; Grb2: growth factor receptor-bound protein 2; JNK: Jun N-terminal kinase/Janus kinase; MAPK: Mitogen-activated protein kinase; MEK: Mitogen ERK; MEKK: Mitogen ERK kinase; MLK: Mixed lineage kinase; PAK: p21 activated kinase; PI3K: phosphatidylinositide-3-kinase; PKC: protein kinase c; Myc: avian myelocytomatosis viral oncogene homologue; PLCγ: Phospholipase C γ; RAF: murine leukemia viral oncogene homologue; Ras: Rat sarcoma viral oncogene homologue; SH: src homology domain; S6K: S6-kinase; Sos: Son of sevenless; STAT: Signal transducer and activator of transcription; TPI2: thiol proteinase inhibitor 2.

marginal efficacy. The consensus that has emerged among investigators is that surmounting the cancer therapeutic problem will be greatly facilitated by an in-depth understanding of the molecular genetics underlying individual malignancies.

Autonomous cell growth resulting in tissue invasion and metastases is the defining feature of all malignant neoplasms (1). Cancers do not necessarily arise solely as a result of an accelerated rate of cell proliferation. Rather, they are the consequence of an imbalance between the rate of cell-cycle progression (cell division) and cell growth (cell mass) on one hand and programmed cell death (apoptosis) on the other. Researchers now recognize that aberrant cellular signal transduction pathways play a vital role in driving this imbalance and hence in malignant transformation (1).

Perhaps one of the most critical groups of signaling molecules involved in normal and abnormal cellular regulation are the tyrosine kinases (2). These proteins constitute a family of enzymes that catalyze the phosphorylation of the tyrosine residues of various target molecules. This process controls fundamental cellular processes including cell cycle, migration, metabolism, proliferation, differentiation, and survival (2). Importantly, several tyrosine kinases are aberrantly expressed in malignancies. The underlying defects may include, but are not limited to, mutation, hybrid gene formation, amplification, and perturbation of transcriptional machinery (3).

In this review, we will highlight the role of select tyrosine kinases—Bcr-Abl, *KIT*, and platelet-derived growth factor receptor (PDGFR)—in the clinical setting. A specific inhibitor (imatinib mesylate) has been developed against these kinases, and this compound demonstrates definitive therapeutic activity. More recently, other kinases, including epidermal growth factor receptor (EGFR) and the vascular endothelial growth factor (VEGF) system, have also been targeted successfully. On the basis of the knowledge gained in the emerging field of molecular cancer therapeutics, scientists are now developing a wealth of new compounds.

STRUCTURE AND FUNCTION OF TYROSINE KINASES: AN OVERVIEW

Tyrosine kinases, enzymes that add a phosphate group to a tyrosine residue in a protein substrate, exist as receptor-coupled forms (the receptor tyrosine kinases) and cytosolic forms (2). Some kinases, such as Abl, may also be nuclear (4–6). Common features of all tyrosine kinases include a separate domain for substrate binding, ATP binding, and catalysis (Figure 1) (2). The latter domain promotes the transfer of the terminal phosphoryl group from ATP to a tyrosine amino group acceptor in a substrate. Autophosphorylation may also occur.

Over 90 tyrosine kinases have been identified, more than half of which are the transmembrane receptor type; the balance are the cytoplasmic nonreceptor type (3). Tyrosine kinase receptors transduce signals from both outside and inside the cell and function as relay points for signaling pathways inside the cell. The cytoplasmic

nonreceptor tyrosine kinases lack a transmembrane segment and generally function downstream of the receptor tyrosine kinases (7).

Receptor tyrosine kinases comprise an extracellular domain containing a ligand-binding site, a single hydrophobic transmembrane α helix, and a cytosolic domain that includes a region with protein-tyrosine kinase activity. Ligand binding causes most receptor tyrosine kinases to dimerize. The protein kinase of the receptor monomer phosphorylates a distinct set of tyrosine residues in the cytosolic domain of its dimer partner (autophosphorylation). Initially, tyrosine residues in the phosphorylation lip near the catalytic site are phosphorylated. This leads to a conformational change that facilitates binding of ATP in some receptors (such as the insulin receptor) and binding of protein substrates in other receptors (such as the fibroblast growth factor receptor). Subsequently, the receptor kinase activity phosphorylates other sites in the cytosolic domain. The resulting phosphotyrosines serve as docking sites for adapter proteins containing src homology 2 (SH2) domains. These adapter proteins can either phosphorylate effector molecules themselves or, if devoid of kinase activity, couple the activated receptors to other components of the signal transduction pathway (2, 7–10) (Figure 1).

Altered tyrosine kinases drive the development of several malignancies. There are four major mechanisms for oncogenic transformation by tyrosine kinases: (*a*) retroviral transduction of a proto-oncogene corresponding to a tyrosine kinase, concomitant with deregulating structural changes (a common transforming mechanism in animals) (11); (*b*) genomic rearrangements, such as chromosomal translocations, which result in oncogenic fusion proteins containing a tyrosine kinase catalytic domain and part of an unrelated protein (e.g., Bcr-Abl in Philadelphia chromosome–positive leukemias); (*c*) gain-of-function mutations or small deletions in tyrosine kinases (e.g., *KIT* in gastrointestinal stromal tumors); and (*d*) tyrosine kinase overexpression resulting from gene amplification (e.g., EGFR in several solid tumors) (3). In general, the transforming effect can be ascribed to enhanced or constitutive kinase activity that escapes normal cellular control mechanisms and induces quantitatively or qualitatively altered downstream signaling. It is now apparent that aberrant kinases are excellent targets for therapeutic intervention.

Development of Tyrosine Kinase Inhibitors: Imatinib Mesylate as a Prototype

Initially, protein kinase enzymes were thought to be poor treatment targets because of their ubiquitous nature and critical role in diverse physiologic processes. However, the advent of imatinib mesylate as a prototype of signal transduction inhibitors (STI) demonstrated that designer tyrosine kinase inhibitors could be specific and effective therapeutic tools (12–42). This is because kinases are notably distinct in how their catalysis is regulated, even though they share catalytic domains conserved in sequence and structure. The ATP binding pocket lies between the two lobes of the kinase fold. This site, together with the less conserved surrounding

pockets, has been the focus of inhibitor design that exploits differences in kinase structure and pliability in order to achieve selectivity. Imatinib mesylate [also known as Gleevec® (USA), Glivec® (Europe), STI 571, or CGP57148] has shown remarkable clinical activity in Philadelphia chromosome–positive leukemias, in gastrointestinal stromal tumors (GIST), and in several unusual tumors with alterations in the PDGF system.

Imatinib mesylate was developed from a lead compound identified in a high-throughput compound screening program searching for protein kinase C and PDGF receptor inhibitors (13). The initial compound was a phenylaminopyrimidine that was modified to increase cellular activity, solubility, and oral bioavailability (16). Imatinib mesylate occupies the nucleotide-binding cleft of the Bcr-Abl protein tyrosine kinase, preventing access of ATP to the substrate and thus competitively inhibiting phosphorylation of downstream effector molecules (14).

In pioneering work, Druker and coworkers demonstrated that imatinib mesylate suppressed proliferation of BCR-ABL–positive chronic myelogenous leukemia (CML) cells in vitro (15). Normal hematopoietic progenitors were mostly unaffected (15). Imatinib mesylate also showed activity against Philadelphia chromosome–positive acute lymphoblastic leukemia (ALL) cells and in in vivo models (17). This compound is also an effective inhibitor of the PDGF receptor tyrosine kinase and kit (CD 117) (stem cell factor receptor) tyrosine kinases (18). Imatinib mesylate is very specific, with 50% inhibiting concentrations (IC50s) of 188 nM for c-Abl, 413 nM for c-Kit, and 386 nM for PDGFR-β, as opposed to IC50s of $>$10,000 nM for most of the other cellular tyrosine kinases (13). These observations laid the groundwork for the use of imatinib mesylate in the clinical setting, with potential for killing tumor cells harboring the target kinases without harm to normal host tissue. Imatinib mesylate shows striking antitumor effects in Bcr-Abl–positive (Philadelphia chromosome–positive) leukemias, GISTs, with activating *KIT*mutations, and in a variety of cancers with alterations in the PDGF system (19–42) (Tables 1 and 2).

Philadelphia Chromosome–Positive BCR-ABL–Positive Leukemias: Clinical and Molecular Features

The Philadelphia chromosome is a shortened chromosome 22. It usually results from a balanced translocate between chromosomes 9 and 22 [t(9:22) (q34;q11)] (43, 44). Philadelphia chromosome–positive leukemias include CML and a subset of acute leukemias, most commonly ALL. The Philadelphia translocation juxtaposes two genes, *BCR* and *ABL*, to form a chimeric *BCR-ABL* gene, located on chromosome 22. The Bcr-Abl protein is a constitutively active tyrosine kinase. This abnormal enzymatic activation is crucial to the oncogenic potential of *BCR-ABL* (45–47).

The natural history of CML exemplifies the process of stepwise tumor progression. There is an inevitable evolution from the early chronic phase to an accelerated phase, which ultimately leads to blast crisis. Though CML is a stem cell disorder,

TABLE 1 Features of tumors successfully targeted by imatinib mesylate

Tumor type	Target	Molecular aberration	Response rate	Comment	Reference
Chronic phase CML	Bcr-Abl	$p210^{Bcr\text{-}Abl}$	CHR > 90%	Responses durable Refer to hematologic response	(36, 39)
Blast phase CML and Philadelphia chromosome-positive acute leukemia	Bcr-Abl	$p210^{Bcr\text{-}Abl}$ $p190^{Bcr\text{-}Abl}$	CHR ≈ 5–20%	Responses short-lived	(19–21)
Gastrointestinal stromal tumor	*KIT*	*KIT* mutation (exons 9, 11)	40–90%, depending on criteria	Responses are durable	(22–24)
Mast Cell Disease	*KIT* or *PDGFR-α*	*FIP1L1-PDGFR* *KIT [Phe522sys]*	≈50%	Patients with *FIP1L1-PDGFR* or *KIT* mutation [*Phe522sys*] respond Patients with *KIT* mutation *AspP816Val* are resistant	(25–28)
Dermatofibrosarcoma protuberans	*PDGF*	*Col1/PDGF*	Anecdotal cases	Molecular aberration involves the PDGF ligand rather than the receptor.	(29, 30)
Hypereosinophilic syndrome	*PDGFR-α*	*FIP1L1-PDGFR*	up to ≈90%	Patients with or without aberrant PDGFR can respond, suggesting that an unidentified, imatinib-mesylate-susceptible tyrosine kinase exists.	(31–34)
Chronic myelomonocytic leukemia	*PDGFR-β*	*TEL-PDGFR*	Anecdotal cases	Rare subset of patients with chronic myelomonocytic leukemia	(35)

Abbreviations: CHR = complete hematologic remission.

TABLE 2 Results of representative studies ofimatinib mesylate in Philadelphia-positive leukemias

Stage/Status of disease	CHR (%)	CCR (%)	Progression free survival at 12 mos (%)	Survival at 18 mos (%)	Comment*	Reference
Chronic Phase CML (previously untreated)	>95	≈75	>95	>95	Markedly superior to standard interferon-α and cytarabine	(36, 37)
Chronic Phase CML (Interferon-α failure)	95	≈40	≈90	>95	Dose = 400 mg per day	(38, 39)
Accelerated Phase CML	≈30–40	15–20	≈60	≈75	Dose = 600 mg/day superior to 400 mg/day	(40)
Myeloid Blast Crisis	≈5–20	5–10	median response duration ~6 months	>20	Dose = 600 mg/day superior to 400 mg/day	(19, 21)
Lymphoid Blast Crisis/ Philadelphia-positive ALL	≈5–20	≈10	Median response duration ~3 months	≈40% at 6 months		(19, 20)
CML relapse post allogeneic transplant						(41, 42)
Chronic phase	≈100	≈40–60	Not stated	≈90–100	Results from one larger retrospective and one smaller study. Numbers dependent on stage of disease	
Blast crisis	≈40	≈20–40		≈10–15		

Abbreviations: ALL = acute lymphoblastic leukemia; CML = chronic myelogenous leukemia; CCR = complete cytogenetic response; CHR = complete hematologic remission.

*Number of patients in most studies ranges from about 100 to more than 1000.

the chronic phase is characterized by neutrophilic leukocytosis and can be easily managed. Managing the chronic phase, however, does not prevent the ineluctable progression toward blast transformation, a stage that resembles an aggressive acute leukemia. Once blast crisis occurs, patients succumb within 6 to 12 months. The phenotype of blast crisis is myeloid in two thirds of patients and lymphoid in up to one third. In contrast, Philadelphia-chromosome-positive ALL is characterized by uncontrolled growth of immature lymphoid cells from the outset. Patients with Philadelphia-chromosome-positive ALL responded poorly to chemotherapy when compared to those ALL patients who do not have the Philadelphia chromosome (48, 49).

The t(9:22) translocation appears to be the initial transforming event in CML (50, 51). However, secondary molecular driving forces are needed for disease progression (52). The constitutively activated tyrosine kinase activity of *BCR-ABL* generates constant activation of downstream signaling pathways, as opposed to the closely regulated Abl tyrosine kinase (53, 54). In this way, Bcr-Abl perturbs myriad cellular functions: (*a*) Ras and PI3K signaling; (*b*) cytoskeletal structures; (*c*) adhesion molecules; (*d*) cell survival/apoptosis; (*e*) growth factor dependence; and (*f*) DNA damage and response processes (47). Consequently, disturbed proliferation and survival of cells results in the chronic phase of CML, and the impact of Bcr-Abl on genomic stability/integrity may underlie progression toward blast crisis (55).

Imatinib Mesylate in Chronic Phase CML

Prior to the discovery of imatinib mesylate, standard treatment of chronic phase CML was based on either interferon-α or allogeneic bone marrow transplant (56, 57). Unfortunately, interferon-α was ineffective in late chronic phase, accelerated phase, and blast crisis. Even in early chronic phase, only a small fraction of patients (5–25%) achieved complete cytogenetic remission (defined as elimination of the Philadelphia chromosome and return to a diploid status, as determined by karyotype analysis of bone marrow metaphases). Allogeneic stem cell transplantation did provide curative therapy, but was limited by donor availability and its significant morbidity/mortality.

A large trial of previously untreated patients with chronic phase CML randomized to either imatinib mesylate or to the prior standard therapy (interferon-α given together with cytosine arabinoside, the IRIS trial) revealed that imatinib mesylate was superior in terms of complete hematological response rates (95% versus 56%) and complete cytogenetic responses (76% versus 15%) (37). In addition, freedom from progression to accelerated phase or blast crisis was 97% in the imatinib mesylate-treated group. A survival difference could not be demonstrated, most likely because of the high crossover rate (58%) from the interferon-α to the imatinib mesylate group (37). Imatinib mesylate was also far better tolerated than interferon-α and cytarabine, with an overall discontinuation rate of only 14% in the imatinib group versus 89% for the interferon group. Molecular remissions were

assessed by the sensitive polymerase chain reaction test that can detect *BCR-ABL* transcripts from one leukemic cell among 10^5 normal cells. Thirty-nine percent of imatinib-treated patients achieved more than a 3 log reduction in *BCR-ABL* transcripts. For these patients, the probability of progression-free survival at 24 months was 100% (36).

Results of imatinib mesylate in patients with chronic phase CML who had failed interferon-α were also impressive. Complete hematological responses were reported in 95% of patients. Major and complete cytogenetic response rates were approximately 60% and 40%, respectively. In general, hematologic responses were observed within days to weeks. Progression-free survival after 18 months follow-up was 89% (39).

Imatinib mesylate is now used as front-line therapy for chronic-phase CML. Complete hematologic remission is expected by three months with major (<35% Philadelphia chromosome–positive metaphases) or complete cytogenetic response by 6–12 months. In patients who do not achieve these milestones, the imatinib mesylate dose can be increased or a different treatment strategy may be considered (58).

Imatinib Mesylate in Accelerated Phase and Blast Crisis of CML

Results of imatinib mesylate therapy in patients with accelerated phase of CML and blast crisis are generally inferior to those observed with chronic phase disease. In patients with accelerated CML, sustained (>4 weeks) complete hematological responses were seen in only 34% of patients. Complete cytogenetic responses were achieved in <20% of patients. Results were slightly better if a higher dose of imatinib mesylate was given (600 mg rather than 400 mg per day) (40).

In myeloid blast crisis, the complete hematological response is about 10% to 20%. Major and complete cytogenetic response rates range from 5% to 16%. Hematological responses are short lived, most patients relapse, and median response duration is only about six months. Higher doses (600 and 800 mg) of imatinib mesylate achieve somewhat higher and longer lasting responses (19, 21). Compared with cytosine arabinoside–based chemotherapy regimens in blast crisis, imatinib mesylate produces similar response rates, but with lower toxicity, lower induction mortality, and better survival (59).

In lymphoid blast crisis and Philadelphia chromosome–positive ALL, imatinib mesylate given at doses of 400 or 600 mg daily yields complete hematologic response rates of about 20% or less and complete cytogenetic remission rates of about 10% (19, 20). Reduction of blast count commonly occurs early, often within one week after treatment start. The duration of responses is unfortunately brief, with a median time to progression of about three months. Most patients who progress will succumb to their disease soon thereafter.

Side effects of imatinib mesylate, especially grade three or four neutropenia and thrombocytopenia, are more frequent in accelerated phase and blast crisis

(20, 21). This is likely a consequence of decreased marrow reserve and progression of underlying disease. Combination therapies of imatinib mesylate with more conventional chemotherapy or other investigational agents are being studied.

Imatinib Mesylate after Allogeneic Stem Cell Transplantation

Imatinib mesylate also exhibits activity in patients who relapse after allogeneic stem cell transplantation. Complete hematological response rates are in the range of 70%. Major and complete cytogenetic responses occur in about 55% and 40% of patients, respectively. More importantly, complete molecular remissions have been observed in about 25% of patients. Response rates in all categories were highest in chronic phase CML and progressively decreased in accelerated and blastic phase (41, 42). Estimated overall survival at two years was 12% in blast crisis and 100% in early chronic phase (42). Thus, imatinib mesylate can be considered an important treatment alternative for CML patients who relapse after allogeneic stem cell transplantation.

Resistance to Imatinib Mesylate

Despite the dramatic success achieved by imatinib mesylate, the issue remains as how to maximize response and defy resistance. Even in early chronic phase CML, not all patients will attain cytogenetic remission. Furthermore, most individuals with blast transformation or Philadelphia chromosome–positive acute leukemia who respond will relapse quickly. Because imatinib mesylate is commonly used as front-line therapy in CML, its impact on long-term survival remains to be seen, and comparison to allogeneic stem cell transplantation warrants full study.

Recent research has revealed mechanisms that mediate resistance. These include upregulation of multi-drug resistance proteins, functional inactivation of imatinib mesylate, *BCR-ABL* gene amplification or mutations, and loss of the Bcr-Abl kinase target (60–62). The most cogent evidence supports a role for mutations in the emergence of resistance. Indeed, mutations in the *BCR-ABL* kinase have been detected in up to 90% of patients who relapsed after initial response (62–64). In some patients, mutations have been present prior to starting treatment and thus, mutated clones were presumably selected by a growth advantage during imatinib mesylate treatment, similar to selection of resistant bacterial clones with antibiotic treatment (65). Alternative innovative approaches that directly interfere with Bcr-Abl function or enhance imatinib mesylate efficacy have therefore been proposed: (*a*) targeting BCR-ABL RNA with antisense oligonucleotides or with ribozymes (66); (*b*) using Bcr fragments as therapy (on the basis of the observation that high levels of Bcr attenuate Bcr-Abl kinase activity) (67); (*c*) treating with molecules, such as tyrphostin, that affect the binding of peptide substrates (rather than ATP) to Bcr-Abl; (*d*) combining imatinib mesylate with other suppressors of signaling (Jak2, Ras) (68, 69); (*e*) administering interferon-α, which has known activity in CML, together with imatinib mesylate; (*f*) using suppressors of nuclear export to entrap Bcr-Abl in the nucleus, where it promotes apoptosis (70); and

(*g*) using dual src-abl inhibitors (such as BMS-354825), which impose less stringent conformational requirements on ABL for kinase inhibition. Indeed, BMS-354825 has proved effective in animal models and clinical trials are underway (72).

The efficacy of these strategies may depend on the mechanism of resistance, which could vary among patients. Although a large body of data implicates *BCR-ABL* mutations in the emergence of imatinib resistance, cells with mutated *BCR-ABL* often do not make up the predominant population in resistant disease. Hence, other mechanisms of resistance must be operative in some individuals. Loss of the Bcr-Abl kinase target or activation of pathways that supplant the role of Bcr-Abl may play a role (71). Approaches that target Bcr-Abl function or levels may be moot for persons in whom molecular pathways other than Bcr-Abl mediate resistance to imatinib mesylate.

PDGFR and Imatinib Mesylate

Recently, imatinib mesylate has shown remarkable activity in certain other hematologic malignancies and solid tumors: idiopathic hypereosinophilic syndrome, eosinophilia-associated chronic myeloid disorder, chronic myelomonocytic leukemia, systemic mast cell disease, atypical CML and dermatofibrosarcoma protuberans (Table 1). The generation of a constitutively active PDGFR tyrosine kinase is the common feature in these responsive tumors. Activation of the receptor can be caused by an aberration in the gene encoding the PDGF receptor or in the gene encoding the *PDGF* ligand.

The PDGFR family consists of the *PDGF-*α and -β receptors, which are tyrosine kinase receptors stimulated by several extracellular *PDGF* ligands. Both receptors have many well-characterized functions and are involved in proliferation, intracellular organization, chemotaxis, apoptosis, as well as oncogenic transformation (for review, see 10) (Figure 1). Imatinib mesylate inhibits the tyrosine kinase enzyme activity of both PDGFR-α and PDGFR-β (18).

Idiopathic Hypereosinophilic Syndrome

Idiopathic hypereosinophilic syndrome and chronic eosinophilic leukemia comprise a spectrum of rare disorders characterized by eosinophil overproduction and clinical symptoms arising from organ involvement. Some patients carry a fusion gene designated FIP1L1-PDFGRA that activates PDGFR-α (31) and is generated by a cryptic interstitial chromosomal deletion on chromosome 4q12. Imatinib mesylate is effective in patients harboring this abnormality, regardless of whether they are classified as hypereosinophilic syndrome or as chronic eosinophilic leukemia. In addition, responders can lack FIP1L1-PDGFR, suggesting that another relevant kinase is present (73). Sustained responses with imatinib mesylate are achieved at doses lower than those needed in CML, i.e., 100 to 400 mg per day, rather than 400 or more mg per day. This finding is consistent with the low IC50 of imatinib mesylate for suppressing FIP1L1-PDGFR kinase ($\approx$3.2 nM) (31) compared with its IC50 for the Bcr-Abl kinase ($\approx$200 nM). Response can be seen within

four weeks. As in CML, new mutations in this fusion gene may lead to resistance. Rarely, patients with systemic mast cell disease also carry a FIP1L1-PDFGR fusion gene; patients harboring this mutation show response to imatinib mesylate treatment (26).

Chronic Myelomonocytic Leukemia

Chronic myelomonocytic leukemia is a myeloproliferative disorder characterized by an increased number of monocytes and granulocytes as well as dysplasia. There is no approved standard therapy for this illness. A small proportion of patients with chronic myelomonocytic leukemia have aberrations involving the PDGFR-β, with constitutive activation of the receptor tyrosine kinase. This aberration is caused by a translocation between chromosomes 5 and 12, t [5;12], creating a fusion gene *ETV6-PDGFRB* (also called *TEL-PDGFRB*). Durable clinical, hematological, cytogenetic, and molecular responses can be achieved in these patients with the use of imatinib mesylate (34, 35).

Atypical CML

In several cases of atypical CML, the *BCR* region is fused to *PDGFR* because of a translocation between chromosomes 4 and 22 instead of the usual t(9:22). Rapid responses to imatinib mesylate have been reported in patients with this variant, demonstrating the activity against the *PDGFR* tyrosine kinase in vivo in these individuals (74).

Dermatofibrosarcoma Protuberans

Dermatofibrosarcoma protuberans is an uncommon, low-grade, fibrohistiocytic tumor of intermediate malignant potential. This neoplasm represents a unique molecular situation where the *PDGF* ligand, rather than the *PDGF* receptor itself, is altered. Patients with dermatofibrosarcoma protuberans harbor a t(17, 22) translocation that generates a *Col1-PDGF* fusion gene (75). Fusion to *Col1* enhances *PDGF* action by allowing constitutive expression of *Col1-PDGF* ligand and constitutive *PDGFR* kinase activation through autocrine stimulation. Exposure of primary cultures of dermatofibrosarcoma protuberans to imatinib mesylate *in vitro* has been shown to inhibit cell growth (76). Further, patients with this tumor respond to imatinib mesylate even in the case of inoperable, metastatic disease (29, 30).

KIT and Gastrointestinal Stromal Tumors (GIST)

GIST are rare mesenchymal tumors arising from the interstitial cells of Cajal in the gastrointestinal tract and abdomen. They represent less than one percent of gastrointestinal tract tumors and a minority of all sarcomas (77). The vast majority of GIST express the type III receptor tyrosine kinase CD117 (*KIT* or stem cell factor receptor) (78). *KIT* resides on chromosome 4 (4q11-q12) and is translated as a

145-kD receptor tyrosine kinase (79, 80). *KIT* is similar in structure and function to the *PDFGR* or *Flt3-receptor*. It is expressed by many cells including hematopoietic progenitor cells, germ cells, and mast cells (81). Physiologic functions of *KIT* include cell survival, proliferation, differentiation, adhesion, and apoptosis (82, 83).

Several activating mutations of *KIT* lead to constitutive, ligand-independent activation of the receptor tyrosine kinase and intracellular downstream pathways including *STAT*, *PI3K* and *MAPK* (82, 84). Therefore, *KIT* expression is thought to represent a crucial step in tumorigenesis. Some GIST tumors lack a mutation in the KIT gene but still possess an activated, phosphorylated *KIT* protein (85). Mechanisms that might account for this finding include other undetected *KIT* mutations, *KIT* ligand up-regulation, *KIT* heterodimerization, or alteration of phosphatases that inhibit *KIT* (85). The natural ligand of *KIT* is stem cell factor. Interestingly, 60–70% of GIST also express the CD34 antigen, a hematopoietic stem cell marker of unknown function (86).

Prior to the availability of imatinib mesylate, prognosis of GIST was grave. Conventional chemotherapy had response rates close to zero. This changed dramatically with the use of imatinib mesylate. The first patient reported had rapidly progressive GIST, resistant to chemotherapy but responded to imatinib mesylate with a complete metabolic response and tumor shrinkage (87). In subsequent large studies for recurrent or advanced GIST, imatinib mesylate exhibited overall response rates (stable disease or partial response) anywhere from >40% (by conventional response criteria) to 85% meaningful clinical responses (22–24, 88). The value of conventional response criteria [assessed by imaging studies, usually in the form of computerized tomography (CT) scans] is in doubt because >90% of treated patients showed clinical benefit, as manifest by long-term relief of cancer-related symptoms. Patients should, therefore, be kept on the medication if they do well symptomatically. Clinical improvement often occurs within one to two days. Remarkably, positron emission tomography (PET) scans demonstrate metabolic turn off of the tumor within several days and are highly predictive of anatomic response (Figure 2). Conventional CT scans, on the other hand, may be misleading. They may demonstrate stability of disease or even disease growth for months, despite clear-cut PET responses. On this basis, Choi and colleagues (89) proposed new CT response criteria, including a greater than 10% decrease in tumor size (rather than the usual greater than 50% decrease) or a greater than 15% decrease in Hounsfield units, a measurement of tumor density on CT scan. Of particular importance is that responses continue to increase with duration of treatment (23). This is in contrast to chemotherapy, in which lack of early response predicts the futility of further treatment. In the large phase III trials, progression-free survival at 12 months was close to 70%, and overall survival was about 85%. Until now, there has been no established dose response difference between 400, 600, and 800 mg imatinib mesylate (22, 90). However in patients failing treatment with 400 mg of imatinib mesylate per day, increase to 800 mg per day of imatinib mesylate can still be effective in up to 7% of patients (J. Trent, unpublished data). Response rate

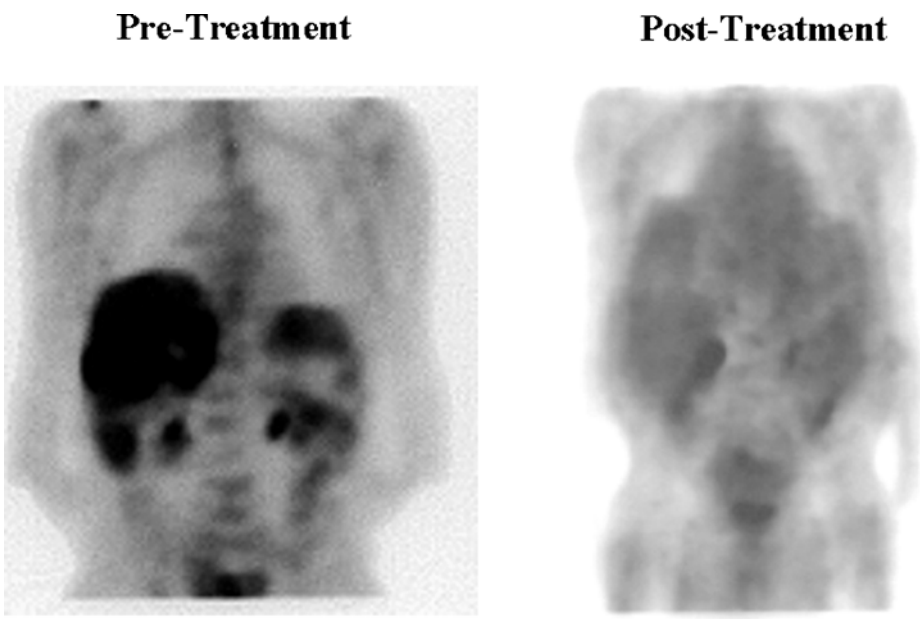

Figure 2 PET scan of a patient with GIST before and after treatment with imatinib mesylate. Positron emission tomography (PET) scan uses a small amount of radioactive glucose [(18F) fluorodeoxyglucose (FDG, FDG-PET)] injected intravenously. This material enriches in areas of increased metabolic activity, such as in tumors. Emitted gamma ray photons are detected with a scanner reflecting cell/tumor metabolism and showing tumor distribution in vivo. PET scans can be used for diagnosis, staging, and monitoring treatment of cancers. This scan shows a GIST patient before and after treatment with imatinib mesylate. Dark areas represent tumor (pretreatment). These areas disappear posttreatment.

appears to correlate with site of mutation. Mutations in exon 11 of *KIT* are more favorable than mutations in exon 9. The least favorable prognostic group in GIST lacks the *KIT* mutation and has no other identifiable mutations (91).

Taken together, these observations suggest that the molecular defect in GIST is inextricably related to tumor response, and that evaluation of response with new targeted therapies may require clinical and imaging endpoints that differ from those established over the years for chemotherapy.

Mast Cell Disease—A Disorder with *KIT* or *PDGFR* Deregulation

Systemic mastocytosis is a clonal neoplasm of the mast cell hematopoietic progenitor. It is a clinically heterogenous disorder with accumulation of mast cells limited to the skin (cutaneous mastocytosis) or involving one or more extracutaneous organs. Mast cell disease is often associated with gain-of-function mutations

involving the tyrosine kinase domain of *KIT* (92). Pardanani et al. (28) prospectively treated 10 adults suffering from symptomatic systemic mast cell disease with imatinib mesylate at a dose of either 100 mg or 400 mg per day. Five of the patients had a measurable response to the drug, four of whom had important mast cell cytoreduction and two of whom had complete clinical and histological remission. Three of the five patients with eosinophilia had major responses. The other two, who did not respond to treatment, were the only patients with the *KIT* Asp816Val mutation. It appears that these KIT mutations confer resistance to imatinib mesylate by interfering with the binding of the drug to the enzymatic site of the *KIT* molecule (27).

To date, two imatinib mesylate–sensitive molecular genetic defects have been identified in mast cell disease. Akin et al. (25) reported a point mutation within the transmembrane segment of *KIT* that resulted in a substitution of a phenylalanine residue by a cysteine at codon 522 in a patient who was amenable to treatment with imatinib mesylate. Pardanani et al. (26) demonstrated that *FIP1L1-PDGFRA* is the therapeutic target of imatinib mesylate in the specific subset of patients with mast cell disease and associated eosinophilia and that virtually all of these patients respond to imatinib.

Adverse Effects of Imatinib Mesylate

Imatinib mesylate is remarkably well tolerated. Predominant adverse effects are usually mild and consist of thrombocytopenia, neutropenia, edema/fluid retention, musculoskeletal pain/muscle cramps, gastrointestinal complaints, fatigue, and headache. Side effects are dose-related and have occurred more frequently with advanced disease. More significant neutropenia and thrombocytopenia occurred in advanced CML states and likely reflect effective inhibition of the leukemic cell clone in the setting of depleted normal progenitors. Fluid retention affects almost half of the patients and is unusual in its periorbital and central abdomen localization. Nausea and gastrointestinal discomfort is uncommon at the 400 mg daily doses, but is frequent at doses of 800 mg per day. Almost one quarter of patients experience myalgias or skin rashes. Overall, fewer than 5% have discontinued treatment because of side effects (93, 94).

Pharmacology of Imatinib Mesylate

Imatinib mesylate has high oral bioavailability. Peak plasma concentrations occur within four hours. Its half-life in humans is from 13 to 16 hours. The drug is metabolized in the liver (primarily via cytochrome P450-3A4). Sufficient plasma concentrations to achieve IC50s can be attained at doses >400 mg once a day. Serum levels of imatinib mesylate range from 1.46–4.6 μM (95). The recommended dose of imatinib mesylate for patients with chronic phase CML is 400 mg by mouth per day. The dose is 600 mg per day for patients in accelerated/blast crisis phase of the disease.The dose can be increased to 800 mg per day based on tolerance and response. Patients with GIST are treated at a recommended dose of

TABLE 3 Tyrosine kinase inhibitors in the clinic

Agent	Target	FDA-approved agents and best established clinical use	Reference
Imatinib mesylate	Bcr-Abl PDGFR Kit	Philadelphia chromosome–positive leukemia* Gastrointestinal stromal tumors* Hypereosinophilic syndrome Mast cell disease Dermatofibrosarcoma protuberans Subset of chronic myelomonocytic leukemia	(28, 35, 37, 73, 93, 94, 96)
Gefitinib	EGFR	Non-small cell lung cancer* Head and neck cancer	(127)
Cetuximab	EGFR	Colorectal cancer* Head and neck cancer	(97, 104)
Bevacizumab	VEGF	Colorectal cancer* Renal cell carcinoma	(99, 100, 133)
Trastuzumab	Erb-2 (Her2/neu)	Breast cancer*	(102, 103)

Abbreviations: EGFR = epidermal growth factor receptor; FDA = Federal Drug Administration; PDGFR = platelet-derived growth factor receptor: VEGF = vascular endothelial growth factor.

*Denotes FDA-approved uses.

400–600 mg/day, but 800 mg/day are easily tolerated and may yield better results. Pediatric doses are calculated by body surface area (93, 94).

Targeting Other Tyrosine Kinase–Related Molecules: Compounds Approved for Clinical Use

Drugs are in development that target aspects of the kinase machinery, from the receptor tyrosine kinases that initiate intracellular signaling, through second messengers involved in signaling cascades, to the kinases that control the cell cycle and govern cellular fate. The vast majority of this plethora of compounds are still in preclinical or early clinical development. However, others have reached the stage of Federal Drug Administration (FDA) approval (Table 3) (28, 35, 37, 73, 93, 94, 96–137). These include molecules that target the EGFR and the VEGF system.

The EGFR Story Unfolds

The EGFR is a family of receptor tyrosine kinases thought to contribute to the formation of many solid tumors (105). This family consists of four different receptor members: (*a*) EGFR, also known as ErbB1 or HER1; (*b*) ErbB2 or Her2/Neu; (*c*) ErbB2 (HER3); and (*d*) ErbB4 (HER4) (2, 106). The ErbB receptors share a similar structure with homology in their kinase domain, but diverge in their extracellular domains and carboxy-terminal end tails (2). The EGFR itself is a 170-kDa transmembrane protein with a single polypeptide chain of 1186 amino acids. It has a hydrophobic transmembrane segment of 23 amino acids attached

to the intracellular domain with tyrosine kinase activity (107, 108). Endogenous activating ligands are EGF, TGF-alpha, heparin-binding EGF, amphiregulin, betacellulin, epiregulin, and many others (105, 109). Transactivation through G-protein coupled receptors and cytokines plays a role (109). ErbB family receptors are widely expressed in all tissues where they regulate diverse functions, including mitogenesis, differentiation, and cell survival (105). Downstream target activation includes PLCγ, Ras-Raf-MEK-MAPK (gene transcription and proliferation), phosphatidylinositol-3 kinase (PI3K)/Akt (cell survival), the tyrosine kinase Scr, the stress-activated protein kinases, PAK-JNKK, JNK, and the signal transducers and activators of transcription (STAT) (110, 111). Of particular importance for the ErbB receptor family is the ability of Erb2/Her2Neu to form heterodimers with the other receptor subunits. ErbB2 does not have an extracellular ligand but is the most oncogenic ErbB receptor-dimer known (109). The EGFR degradation constitutes an important regulatory mechanism. After ligand binding, the receptor is internalized, with signal termination often within seconds, and either further endocytotic degradation or recycling of receptor components to the cell surface for repeated signaling (112).

Mechanisms mediating transformation include receptor overexpression, gene amplification, activating mutations, alterations in the dimerization process, and structural rearrangements (reviewed and referenced in 105). Furthermore activation of autocrine growth factor loops (113) and deficiency of specific phosphatases may be of importance, as well. Receptor and ligand overexpression and gene amplification are the common causes of oncogenic transformation (114). Indeed, the rationale for targeting the EGF receptor tyrosine kinases is based on the receptor overexpression discerned in many human malignancies including colorectal, head and neck, esophageal, ovarian, cervical, breast, endometrial, and nonsmall cell lung cancer (105, 115, 116).

Several strategies to interfere with aberrant EGFR signaling have emerged. The most successful in the clinic are antibodies that block the extracellular ligand-binding site (preventing ligand activation of the receptor) and small molecule tyrosine kinase inhibitors that suppress the intracellular tyrosine kinase. To date, the small molecule tyrosine kinase inhibitors Gefitinib (ZD1839, Iressa®) (117) and the antibody Cetuximab (118) have been approved by the FDA for use in lung and colorectal cancer, respectively. Trastuzumab, which targets Erb-2 (Her2/neu), is approved for treatment of Her2/neu-positive breast cancer. Numerous other inhibitors of the EGFR machinery are in development (reviewed in 119).

Initially, the modest response rates to some of these compounds were considered disappointing. However, recent data demonstrate that it is possible to identify subsets of patients whose tumors have mutations in the targeted kinase that confer profound susceptibility to the inhibitor.

Trastuzumab (Herceptin®)

The approval of Trastuzumab in 1998 for the treatment of breast cancer is a milestone in the field of EGFR-directed therapy. It is now used worldwide for breast cancer management.

Her2/neu acts as a major signaling partner for other EGFR family members by forming heterodimers with potent signaling activity leading to proliferative and antiapoptotic effects. Her2/neu is amplified/overexpressed in about 30% of breast cancers, and correlates with a poor outcome (101, 120). Trastuzumab is an anti-ErbB-2 receptor (Her2/neu) humanized monoclonal antibody with benefit in Her2/neu-positive metastatic breast cancer as a single agent (102). Compared to chemotherapy alone, it demonstrates statistically significant increases in response rate, time to progression, and survival time when combined with chemotherapy (103). Benefit from Trastuzunab is only derived in Her2/neu positive patients, however, so selection of the study population for new targeted agents is very important.

Gefitinib (Iressa®)

Gefitinib is an oral, highly bioavailable, EGFR-specific, anilinoquinazoline, small-molecule inhibitor. It binds to the ATP site of the EGFR tyrosine kinase domain with approximately 100-fold increased affinity compared to other kinases and reversibly inhibits autophosphorylation of the receptor by competitively blocking access of ATP to the EGFR kinase domain (121, 122), which prevents downstream kinase activation. The IC50 for the inhibition of autophosphorylation of the EGFR/Her1 receptor in intact cells is 0.033 μM (123). In preclinical models, gefitinib inhibited the growth of multiple cell lines and mouse tumor xenografts in a dose-dependent manner and was synergistic and additive with chemotherapeutic agents (platinum, taxanes, etoposide), radiation therapy, as well as with the monoclonal antibody Trastuzumab (124–126).

In patients with nonsmall cell lung cancer, there was no synergism when gefitinib was administered with cytotoxic agents, according to two large randomized trials (INTACT-1 and INTACT-2) (134, 135). The combination of gefitinib with radiation therapy might be more promising (124). Even so, gefitinib is now approved for single-agent, third-line therapy of patients with nonsmall cell lung cancer who have failed chemotherapy, on the basis of a response rate of about 10% (127). This modest response rate was initially considered disappointing, and the lack of a correlation between response and EGFR overexpression was frustrating (128). However, a recent discovery indicates that activating mutations in the ATP-binding pocket of the EGFR kinase domain confers susceptibility to gefitinib in nonsmall cell lung cancer, hence allowing identification of subgroups of responsive patients (137).

Gefitinib is well tolerated. The most common side effects are skin rash and gastrointestinal complaints. An oral dose of 250 mg of gefitinib per day is recommended. Steady-state concentrations are achieved in most patients at seven days. Gefitinib can be used in extensively pretreated patients or those with poor performance status, for whom a therapy with a low toxicity profile is needed.

Erlotinib (Tarceva™)

A second small-molecule EGFR inhibitor—erlotinib, Tarceva™, OSI-774—demonstrates response rates of 10% to 19% as a single agent in patients with

nonsmall cell lung cancer who failed chemotherapy (129). However, large randomized trials testing erlotinib in combination with chemotherapy in this disease did not show any benefit derived from the addition of erlotinib (R. Herbst, personal communication). Single agent Tarceva™ in patients with advanced NSCLC failing standard therapy has recently shown to have a survival advantage in a study with 751 patients from Canada (F. Shepherd, Proc. ASW, June 2004).

Cetuximab (C-225) (Erbitux®)

Cetuximab is a chimeric, recombinant humanized monoclonal antibody to the external EGFR/ErbB1 domain. It prevents receptor autophosphorylation and leads to EGFR internalization with subsequent receptor degradation (130). This antibody has nonlinear pharmacokinetics, and clinically effective doses range between 200 and 400 mg/m^2 given intravenously. Cetuximab was recently FDA approved for use in combination with irinotecan for the treatment of patients with EGFR-expressing, metastatic colorectal carcinoma who are refractory to irinotecan-based chemotherapy. Combinations of cetuximab with irinotecan yielded a 23% overall response rate and median time to disease progression of four months (97). Cetuximab alone resulted in about a 10% overall response rate (98). The most common side effects of cetuximab were acneiform skin rash and folliculitis. Severe hypersensitivity reactions were rare.

Angiogenesis: Targeting the Vascular System

Because tumor progression depends on the formation of new blood vessels, blocking angiogenesis is an appealing approach to anticancer therapy. VEGF is a key angiogenic factor. It binds to the VEGF1 and 2 receptors on endothelial and other cells. Binding activates the intracellular pathways necessary for physiologic and tumor angiogenesis (131). Current research is directed to agents that target this molecule or its receptor. Recently, Bevacizumab, which targets the VEGF ligand, has been approved for clinical use in colorectal cancer.

Bevacizumab (Avastin®)

Bevacizumab is a humanized monoclonal antibody. It binds and inhibits the VEGF growth factor ligand. A phase III trial involving 815 patients established Bevacizumab as the first angiogenesis-targeted agent to improve overall survival in patients with metastatic colorectal cancer and lead to FDA approval. The addition of Bevacizumab in the frontline setting to a regimen containing multiagent chemotherapy (irinotecan, 5-fluoruracil, and leucovorin) improved overall survival from 15.6 to 20.3 months and progression-free survival from 6.4 to 10.6 months when compared to the chemotherapy arm (99). These results, although modest, were statistically significant. Bevacizumab has also been tested alone and/or in combination with different conventional cytotoxic agents in several malignancies (i.e., breast cancer, renal cell cancer, and nonsmall cell lung cancer). Overall results were disappointing and few partial tumor regressions were observed (132). Promising results have been seen in renal cell cancer, however (133).

Bevacizumab is administered intravenously at doses of 5–10 mg/kg every two weeks. It is well tolerated. Side effects include headache/migraine, proteinuria, hypertension, and rare cases of hemorrhage, thromboembolic events, and gastrointestinal perforations (100).

SUMMARY: THE STATE OF THE ART

Certain classes of signaling proteins and pathways are frequently altered by oncogenic mutations. Molecules governing extracellular growth, differentiation, and developmental signals, in particular, are often mutated in cancers. Tyrosine kinases are especially important in this respect. These enzymes are pivotal regulators of the signal transduction pathways that mediate development and intracellular communication. Their activity is normally tightly controlled. Perturbation of tyrosine kinase signaling by mutations and other genetic alterations drive malignant transformation.

In principle, for tyrosine kinases involved in cancer, oncogenic deregulation results from alteration of one of several of the auto-control mechanisms that ensure the normal repression of catalytic domains. A little more than half of the known tyrosine kinase receptors have been found repeatedly in either mutated or overexpressed forms associated with (human) malignancies, including sporadic cases. In addition, many of the cytoplasmic tyrosine kinases are also disturbed in cancer, either directly through mutation or indirectly via other genetic driving forces.

Imatinib mesylate, a potent inhibitor of the Bcr-Abl, Kit, and PDGFR kinases, has enjoyed great success in treating tumors, including those that are not susceptible to standard chemotherapy. Response rates of more than 80%–90% can be achieved with little toxicity in some malignancies bearing the proper targets. On the other hand, the use of imatinib mesylate in the treatment of neoplasms in which the pathogenesis is not clearly dependent on activation of a susceptible tyrosine kinase has been disappointing. Even so, there may be other imatinib-sensitive tumors (or subsets of tumors), in addition to CML, GIST, chronic myelomonocytic leukemia, mast cell disease, hypereosinophilic syndrome, and dermatofibrosarcoma protuberans, in which occult activation of the Abl, *KIT*, or *PDGFR* tyrosine kinases exists or in which there is activation of an undiscovered kinase. As an example, some patients with hypereosinophilic syndrome without an obvious target kinase aberration can respond to imatinib mesylate in a manner similar to those with *PDGFR* abnormalities.

Other inhibitors of specific tyrosine kinases have been approved for clinical use, albeit with initial response rates that pale in comparison with those of imatinib mesylate. Antibodies and small-molecule inhibitors of the EGFR and VEGF pathways are now applied in the management of patients suffering from breast cancer, colorectal cancer, and lung cancer. The modest response rates attained with these drugs are almost certainly due to the complex molecular heterogeneity of many solid tumors. Indeed, recent discoveries indicate that the presence of activating mutations in the EGFR allows identification of a subset of

patients with lung cancer in whom EGFR inhibitors can elicit dramatic responses (137).

A synthesis of the knowledge gleaned from the clinical application of tyrosine kinase inhibitors indicates that a new paradigm for cancer therapy is emerging. Optimal exploitation of targeted designer drugs as part of the oncology arsenal mandates that treatment should be based on the molecular fingerprint of the tumor rather than its anatomic locale.

WHAT DOES THE FUTURE HOLD?

Several vital lessons have been learned during the process of developing the tyrosine kinase inhibitor, imatinib mesylate, and the EGFR inhibitors. First, it is now clear that designer compounds can target even ubiquitously expressed families of proteins in a specific manner. Second, tumors that are highly resistant to conventional agents can be exquisitely sensitive to a rationally targeted agent. Third, with the use of such agents, dramatic and durable responses can be achieved without significant host toxicity. Fourth, proteins considered predominantly in the context of the hematologic system (i.e., *PDGFR* or stem cell factor receptor KIT) may have a profound contribution to solid tumors and vice versa. Fifth, to date, it appears that activating mutations in kinases, rather than simple overexpression, confers susceptibility to kinase inhibitors.

Future clinical trials may need to categorize patients by their molecular genetics (e.g., p53 mutation-positive cancer for a therapy directed against the p53 tumor suppressor gene) rather than by the disease site of their illness (breast cancer or colorectal cancer). Such a nosology would represent a significant paradigm shift in that clinical trials could be designed so that patients are enrolled based exclusively on the molecular defect in their tumor. This paradigm also recognizes that classification of tumors based on anatomic location (e.g., lung cancer or sarcoma) belies a complicated underlying molecular heterogeneity that, if unrecognized, may obscure response rates. For instance, GIST comprise only a minority of sarcomas, and these are the only sarcomas that demonstrate profound sensitivity to imatinib mesylate. Similarly, the 10% response to EGFR inhibitors in lung cancer was initially considered a failure or, at best, a marginal success, despite the fact that some patients with chemotherapy-refractory disease demonstrated profound responses. This changed with the discovery that the subset of responders could be identified on the basis that their neoplasms harbor an activating EGFR mutation (137). It is likely that many other cancers, including breast, colorectal, and others, are comprised of numerous small-molecular subsets, and that successful treatment will require precise pinpointing and exploitation of molecular defects as targets for therapy.

The field of molecular cancer therapeutics is now advancing rapidly. Indeed, the time from the discovery of the activating *KIT* mutation in GIST tumors (138) to the remarkable reversal of the hopeless prognosis for this disease via treatment with imatinib mesylate (24) was only three years. Even considering that some

kinase inhibitors may have serious limitations in their salutary activity, our current successes almost certainly represent the tip of the therapeutic iceberg for targeted treatments.

The *Annual Review of Pharmacology and Toxicology* is online at http://pharmtox.annualreviews.org

LITERATURE CITED

1. Hanahan D, Weinberg RA. 2000. The hallmarks of cancer. *Cell* 100:57–70
2. Schlessinger J. 2000. Cell signaling by receptor tyrosine kinases. *Cell* 103:211–25
3. Blume-Jensen P, Hunter T. 2001. Oncogenic kinase signalling. *Nature* 411:355–65
4. Van Etten RA, Jackson P, Baltimore D. 1989. The mouse type IV c-abl gene product is a nuclear protein, and activation of transforming ability is associated with cytoplasmic localization. *Cell* 58:669–78
5. Wetzler M, Talpaz M, Van Etten RA, Hirsh-Ginsberg C, Beran M, Kurzrock R. 1993. Subcellular localization of Bcr, Abl, and Bcr-Abl proteins in normal and leukemic cells and correlation of expression with myeloid differentiation. *J. Clin. Invest.* 92:1925–39
6. Wetzler M, Talpaz M, Yee G, Stass SA, Van Etten RA, et al. 1995. Cell cycle-related shifts in subcellular localization of BCR: association with mitotic chromosomes and with heterochromatin. *Proc. Natl. Acad. Sci. USA* 92:3488–92
7. Hubbard SR, Till JH. 2000. Protein tyrosine kinase structure and function. *Annu. Rev. Biochem.* 69:373–98
8. Ullrich A, Schlessinger J. 1990. Signal transduction by receptors with tyrosine kinase activity. *Cell* 61:203–12
9. Heldin CH. 1995. Dimerization of cell surface receptors in signal transduction. *Cell* 80:213–23
10. Heldin CH, Westermark B. 1999. Mechanism of action and in vivo role of platelet-derived growth factor. *Physiol. Rev.* 79:1283–316
11. Miller WE, Raab-Traub N. 1999. The EGFR as a target for viral oncoproteins. *Trends Microbiol.* 7:453–58
12. Druker BJ, Lydon NB. 2000. Lessons learned from the development of an abl tyrosine kinase inhibitor for chronic myelogenous leukemia. *J. Clin. Invest.* 105:3–7
13. Manley PW, Cowan-Jacob SW, Buchdunger E, Fabbro D, Fendrich G, et al. 2002. Imatinib: a selective tyrosine kinase inhibitor. *Eur. J. Cancer* 38 (Suppl 5.): S19–S27
14. Savage DG, Antman KH. 2002. Imatinib mesylate—a new oral targeted therapy. *New Engl. J. Med.* 346:683–93
15. Druker BJ, Tamura S, Buchdunger E, Ohno S, Segal GM, et al. 1996. Effects of a selective inhibitor of the Abl tyrosine kinase on the growth of Bcr-Abl positive cells. *Nat. Med.* 2:561–66
16. Schindler T, Bornmann W, Pellicena P, Miller WT, Clarkson B, Kuriyan J. 2000. Structural mechanism for STI-571 inhibition of abelson tyrosine kinase. *Science* 289:1857–59
17. le Coutre P, Mologni L, Cleris L, Marchesi E, Buchdunger E, et al. 1999. In vivo eradication of human BCR/ABL-positive leukemia cells with an ABL kinase inhibitor. *J. Natl. Cancer Inst.* 91:163–68
18. Buchdunger E, Cioffi CL, Law N, Stover D, Ohno-Jones S, et al. 2000. Abl protein-tyrosine kinase inhibitor STI571 inhibits in vitro signal transduction mediated by c-kit and platelet-derived growth factor receptors. *J. Pharmacol. Exp. Ther.* 295:139–45
19. Druker BJ, Sawyers CL, Kantarjian H,

Resta DJ, Reese SF, et al. 2001. Activity of a specific inhibitor of the BCR-ABL tyrosine kinase in the blast crisis of chronic myeloid leukemia and acute lymphoblastic leukemia with the Philadelphia chromosome. *N. Engl. J. Med.* 344:1038–42

20. Ottmann OG, Druker BJ, Sawyers CL, Goldman JM, Reiffers J, et al. 2002. A phase 2 study of imatinib in patients with relapsed or refractory Philadelphia chromosome-positive acute lymphoid leukemias. *Blood* 100:1965–71
21. Sawyers CL, Hochhaus A, Feldman E, Goldman JM, Miller CB, et al. 2002. Imatinib induces hematologic and cytogenetic responses in patients with chronic myelogenous leukemia in myeloid blast crisis: results of a phase II study. *Blood* 99:3530–39
22. Benjamin RS, Rankin C, Fletcher CD, Demetri GD. 2003. Phase III dose-randomized study of imatinib mesylate (STI571) for GIST: Intergroup S0033 early results. *Proc. Am. Soc. Clin. Oncol.* 22:3271 (Abstr.)
23. Blanke CD, Joensuu H, Demetri GD, Heinrich P, von Mehren M. 2003. Long-term follow up of advanced gastrointestinal stomal tumor (GIST) patients treated with imatinib mesylate. *Proc. Am. Soc. Clin. Oncol.* 22:2 (Abstr.)
24. Demetri GD, von Mehren M, Blanke CD, Van den Abbeele AD, Eisenberg B, et al. 2002. Efficacy and safety of imatinib mesylate in advanced gastrointestinal stromal tumors. *N. Engl. J. Med.* 347:472–80
25. Akin C, Fumo G, Yavuz AS, Lipsky PE, Neckers L, Metcalfe DD. 2004. A novel form of mastocytosis associated with a transmembrane c-kit mutation and response to imatinib. *Blood* 103:3222–25
26. Pardanani A, Ketterling RP, Brockman SR, Flynn HC, Paternoster SF, et al. 2003. CHIC2 deletion, a surrogate for FIP1L1-PDGFRA fusion, occurs in systemic mastocytosis associated with eosinophilia and predicts response to imatinib mesylate therapy. *Blood* 102:3093–96
27. Ma Y, Zeng S, Metcalfe DD, Akin C, Dimitrijevic S, et al. 2002. The c-KIT mutation causing human mastocytosis is resistant to STI571 and other KIT kinase inhibitors; kinases with enzymatic site mutations show different inhibitor sensitivity profiles than wild-type kinases and those with regulatory-type mutations. *Blood* 99:1741–44
28. Pardanani A, Elliott M, Reeder T, Li CY, Baxter EJ, et al. 2003. Imatinib for systemic mast-cell disease. *Lancet* 362:535–36
29. Maki RG, Awan RA, Dixon RH, Jhanwar S, Antonescu CR. 2002. Differential sensitivity to imatinib of 2 patients with metastatic sarcoma arising from dermatofibrosarcoma protuberans. *Int. J. Cancer* 100:623–26
30. Rubin BP, Schuetze SM, Eary JF, Norwood TH, Mirza S, et al. 2002. Molecular targeting of platelet-derived growth factor B by imatinib mesylate in a patient with metastatic dermatofibrosarcoma protuberans. *J. Clin. Oncol.* 20:3586–91
31. Cools J, DeAngelo DJ, Gotlib J, Stover EH, Legare RD, et al. 2003. A tyrosine kinase created by fusion of the PDGFRA and FIP1L1 genes as a therapeutic target of imatinib in idiopathic hypereosinophilic syndrome. *N. Engl. J. Med.* 348:1201–14
32. Cortes J, Ault P, Koller C, Thomas D, Ferrajoli A, et al. 2003. Efficacy of imatinib mesylate in the treatment of idiopathic hypereosinophilic syndrome. *Blood* 101:4714–16
33. Gleich GJ, Leiferman KM, Pardanani A, Tefferi A, Butterfield JH. 2002. Treatment of hypereosinophilic syndrome with imatinib mesilate. *Lancet* 359:1577–78
34. Pardanani A, Tefferi A. 2004. Imatinib therapy for hypereosinophilic syndrome and eosinophilia-associated myeloproliferative disorders. *Leuk. Res.* 28 (Suppl. 1):47–52
35. Apperley JF, Gardembas M, Melo JV, Russell-Jones R, Bain BJ, et al. 2002.

Response to imatinib mesylate in patients with chronic myeloproliferative diseases with rearrangements of the platelet-derived growth factor receptor beta. *N. Engl. J. Med.* 347:481–87

36. Hughes TP, Kaeda J, Branford S, Rudzki Z, Hochhaus A, et al. 2003. Frequency of major molecular responses to imatinib or interferon alfa plus cytarabine in newly diagnosed chronic myeloid leukemia. *N. Engl. J. Med.* 349:1423–32
37. O'Brien SG, Guilhot F, Larson RA, Gathmann I, Baccarani M, et al. 2003. Imatinib compared with interferon and low-dose cytarabine for newly diagnosed chronic-phase chronic myeloid leukemia. *N. Engl. J. Med.* 348:994–1004
38. Cortes J, Giles F, O'Brien S, Thomas D, Garcia-Manero G, et al. 2003. Result of high-dose imatinib mesylate in patients with Philadelphia chromosome-positive chronic myeloid leukemia after failure of interferon-alpha. *Blood* 102:83–86
39. Kantarjian H, Sawyers C, Hochhaus A, Guilhot F, Schiffer C, et al. 2002. Hematologic and cytogenetic responses to imatinib mesylate in chronic myelogenous leukemia. *N. Engl. J. Med.* 346:645–52
40. Talpaz M, Silver RT, Druker BJ, Goldman JM, Gambacorti-Passerini C, et al. 2002. Imatinib induces durable hematologic and cytogenetic responses in patients with accelerated phase chronic myeloid leukemia: results of a phase 2 study. *Blood* 99:1928–37
41. Kantarjian HM, O'Brien S, Cortes JE, Giralt SA, Rios MB, et al. 2002. Imatinib mesylate therapy for relapse after allogeneic stem cell transplantation for chronic myelogenous leukemia. *Blood* 100:1590–95
42. Olavarria E, Ottmann OG, Deininger M, Clark RE, Bandini G, et al. 2003. Response to imatinib in patients who relapse after allogeneic stem cell transplantation for chronic myeloid leukemia. *Leukemia* 17:1707–12
43. Rowley JD. 1973. A new consistent chromosomal abnormality in chronic myelogenous leukaemia identified by quinacrine fluorescence and Giemsa staining. *Nature* 243:290–93
44. Nowell PC, Hungerford DA. 2004. A minute chromosome in human chronic granulocytic leukemia. *Science* 132:1497 (Abstr.)
45. Kloetzer W, Kurzrock R, Smith L, Talpaz M, Spiller M, et al. 1985. The human cellular abl gene product in the chronic myelogenous leukemia cell line K562 has an associated tyrosine protein kinase activity. *Virology* 140:230–38
46. Kurzrock R, Shtalrid M, Romero P, Kloetzer WS, Talpas M, et al. 1987. A novel c-abl protein product in Philadelphia-positive acute lymphoblastic leukaemia. *Nature* 325:631–35
47. Kurzrock R, Kantarjian HM, Druker BJ, Talpaz M. 2003. Philadelphia chromosome-positive leukemias: from basic mechanisms to molecular therapeutics. *Ann. Intern. Med.* 138:819–30
48. Sawyers CL. 1999. Chronic myeloid leukemia. *N. Engl. J. Med.* 340:1330–40
49. Garcia-Manero G, Faderl S, O'Brien S, Cortes J, Talpaz M, Kantarjian HM. 2003. Chronic myelogenous leukemia: a review and update of therapeutic strategies. *Cancer* 98:437–57
50. Kelliher MA, McLaughlin J, Witte ON, Rosenberg N. 1990. Induction of a chronic myelogenous leukemia-like syndrome in mice with v-abl and BCR/ABL. *Proc. Natl. Acad. Sci. USA* 87:6649–53
51. Heisterkamp N, Jenster G, ten Hoeve J, Zovich D, Pattengale PK, Groffen J. 1990. Acute leukaemia in bcr/abl transgenic mice. *Nature* 344:251–53
52. Guerrasio A, Serra A, Gottardi E, Cilloni D, Vischia F, et al. 1997. Molecular events in chronic myeloid leukemia progression. *Leukemia* 11 (Suppl. 3):519–21
53. Konopka JB, Watanabe SM, Witte ON. 1984. An alteration of the human c-abl protein in K562 leukemia cells unmasks

associated tyrosine kinase activity. *Cell* 37:1035–42

54. Davis RL, Konopka JB, Witte ON. 1985. Activation of the c-abl oncogene by viral transduction or chromosomal translocation generates altered c-abl proteins with similar in vitro kinase properties. *Mol. Cell Biol.* 5:204–13
55. Laurent E, Mitchell DL, Estrov Z, Lowery M, Tucker SL, et al. 2003. Impact of p210(Bcr-Abl) on ultraviolet C wavelength–induced DNA damage and repair. *Clin. Cancer Res.* 9:3722–30
56. Sawyers CL. 1999. Chronic myeloid leukemia. *N. Engl. J. Med.* 340:1330–13
57. Talpaz M, Kantarjian HM, McCredie K, Trujillo JM, Keating MJ, Gutterman JU. 1986. Hematologic remission and cytogenetic improvement induced by recombinant human interferon alpha A in chronic myelogenous leukemia. *N. Engl. J. Med.* 314:1065–69
58. Kantarjian H, Talpaz M, O'Brien S, Giles F, Rios MB, et al. 2003. Prediction of initial cytogenetic response for subsequent major and complete cytogenetic response to imatinib mesylate therapy in patients with Philadelphia chromosome-positive chronic myelogenous leukemia. *Cancer* 97:2225–28
59. Kantarjian HM, Cortes J, O'Brien S, Giles FJ, Albitar M, et al. 2002. Imatinib mesylate (STI571) therapy for Philadelphia chromosome-positive chronic myelogenous leukemia in blast phase. *Blood* 99:3547–53
60. Weisberg E, Griffin JD. 2000. Mechanism of resistance to the ABL tyrosine kinase inhibitor STI571 in BCR/ABL-transformed hematopoietic cell lines. *Blood* 95:3498–505
61. Gorre ME, Mohammed M, Ellwood K, Hsu N, Paquette R, et al. 2001. Clinical resistance to STI-571 cancer therapy caused by BCR-ABL gene mutation or amplification. *Science* 293:876–80
62. Branford S, Rudzki Z, Walsh S, Grigg A, Arthur C, et al. 2002. High frequency of point mutations clustered within the adenosine triphosphate-binding region of BCR/ABL in patients with chronic myeloid leukemia or Ph-positive acute lymphoblastic leukemia who develop imatinib (STI571) resistance. *Blood* 99:3472–75D
63. Hochhaus A, Kreil S, Corbin AS, La Rosee P, Muller MC, et al. 2002. Molecular and chromosomal mechanisms of resistance to imatinib (STI571) therapy. *Leukemia* 16:2190–96
64. Shah NP, Nicoll JM, Nagar B, Gorre ME, Paquette RL, et al. 2002. Multiple BCR-ABL kinase domain mutations confer polyclonal resistance to the tyrosine kinase inhibitor imatinib (STI571) in chronic phase and blast crisis chronic myeloid leukemia. *Cancer Cell.* 2:117–25
65. Roche-Lestienne C, Soenen-Cornu V, Grardel-Duflos N, Lai JL, Philippe N, et al. 2002. Several types of mutations of the Abl gene can be found in chronic myeloid leukemia patients resistant to STI571, and they can pre-exist to the onset of treatment. *Blood* 100:1014–18
66. James HA, Gibson I. 1998. The therapeutic potential of ribozymes. *Blood* 91:371–82
67. Wu Y, Ma G, Lu D, Lin F, Xu HJ, et al. 1999. Bcr: a negative regulator of the Bcr-Abl oncoprotein. *Oncogene* 18:4416–24
68. Sun X, Layton JE, Elefanty A, Lieschke GJ. 2001. Comparison of effects of the tyrosine kinase inhibitors AG957, AG490, and STI571 on BCR-ABL–expressing cells, demonstrating synergy between AG490 and STI571. *Blood* 97:2008–15
69. Beaupre DM, Kurzrock R. 1999. RAS and leukemia: from basic mechanisms to gene-directed therapy. *J. Clin. Oncol.* 17:1071–79
70. Vigneri P, Wang JY. 2001. Induction of apoptosis in chronic myelogenous leukemia cells through nuclear entrapment of BCR-ABL tyrosine kinase. *Nat. Med.* 7:228–34
71. Kurzrock R, Talpaz M, Li N, Estrov

Z. 2003. Distinct biological impact of dephosphorylation vs downregulation of p210 BCR-Abl: Implication for imatinib mesylate response and resistance. *Am. Soc. Hematol.* 102:264 (Abstr.)

72. Shah NP, Tran C, Lee FY, Chen P, Norris D, Sawyers CL. 2004. Overriding imatinib resistance with a novel ABL kinase inhibitor. *Science* 305:399–401
73. Gotlib J, Cools J, Malone JM III, Schrier SL, Gilliland DG, Coutre SE. 2004. The FIP1L1-PDGFR{alpha} fusion tyrosine kinase in hypereosinophilic syndrome and chronic eosinophilic leukemia: implications for diagnosis, classification, and management. *Blood* 103:2879–91
74. Garcia JL, Font DM, Hernandez JM, Queizan JA, Gutierrez NC, et al. 2003. Imatinib mesylate elicits positive clinical response in atypical chronic myeloid leukemia involving the platelet-derived growth factor receptor beta. *Blood* 102: 2699–700
75. Shimizu A, O'Brien KP, Sjoblom T, Pietras K, Buchdunger E, et al. 1999. The dermatofibrosarcoma protuberans-associated collagen type Ialpha1/platelet-derived growth factor (PDGF) B-chain fusion gene generates a transforming protein that is processed to functional PDGF-BB. *Cancer Res.* 59:3719–23
76. Sjoblom T, Shimizu A, O'Brien KP, Pietras K, Dal Cin P, et al. 2001. Growth inhibition of dermatofibrosarcoma protuberans tumors by the platelet-derived growth factor receptor antagonist STI571 through induction of apoptosis. *Cancer Res.* 61:5778–83
77. Berman J, O'Leary TJ. 2001. Gastrointestinal stromal tumor workshop. *Hum. Pathol.* 32:578–82
78. Duffaud F, Blay JY. 2003. Gastrointestinal stromal tumors: biology and treatment. *Oncology* 65:187–97
79. Blume-Jensen P, Claesson-Welsh L, Siegbahn A, Zsebo KM, Westermark B, Heldin CH. 1991. Activation of the human c-kit product by ligand-induced dimerization mediates circular actin reorganization and chemotaxis. *EMBO J.* 10:4121–28
80. Majumder S, Brown K, Qiu FH, Besmer P. 1988. c-kit protein, a transmembrane kinase: identification in tissues and characterization. *Mol. Cell Biol.* 8:4896–903
81. Turner AM, Zsebo KM, Martin F, Jacobsen FW, Bennett LG, Broudy VC. 1992. Nonhematopoietic tumor cell lines express stem cell factor and display c-kit receptors. *Blood* 80:374–81
82. Heinrich MC, Rubin BP, Longley BJ, Fletcher JA. 2002. Biology and genetic aspects of gastrointestinal stromal tumors: KIT activation and cytogenetic alterations. *Hum. Pathol.* 33:484–495
83. Taylor ML, Metcalfe DD. 2000. Kit signal transduction. *Hematol. Oncol. Clin. North Am.* 14:517–35
84. Sattler M, Salgia R. 2004. Targeting c-Kit mutations: basic science to novel therapies. *Leuk. Res.* 28 (Suppl. 1):11–20
85. Rubin BP, Singer S, Tsao C, Duensing A, Lux ML, et al. 2001. KIT activation is a ubiquitous feature of gastrointestinal stromal tumors. *Cancer Res.* 61:8118–21
86. Miettinen M, Virolainen M, Maarit SR. 1995. Gastrointestinal stromal tumors–value of CD34 antigen in their identification and separation from true leiomyomas and schwannomas. *Am. J. Surg. Pathol.* 19:207–16
87. Joensuu H, Roberts PJ, Sarlomo-Rikala M, Andersson LC, Tervahartiala P, et al. 2001. Effect of the tyrosine kinase inhibitor STI571 in a patient with a metastatic gastrointestinal stromal tumor. *N. Engl. J. Med.* 344:1052–56
88. Verweij J, van Oosterom A, Blay JY, Judson I, Rodenhuis S, et al. 2003. Imatinib mesylate (STI-571 Glivec, Gleevec) is an active agent for gastrointestinal stromal tumours, but does not yield responses in other soft-tissue sarcomas that are unselected for a molecular target. Results from an EORTC Soft Tissue and Bone Sarcoma Group phase II study. *Eur. J. Cancer* 39:2006–11

89. Choi H, Charnsangavej C, Macapinlac HA, Burgess MA, Patel SR, et al. 2003. Correlation of computerized tomography (CT) and proton emission tomography (PET) in patients with metastatic GIST treated at a single institution with imatinib mesylate. *Proc. Am. Soc. Clin. Oncol.* 22:3290 (Abstr.)
90. Verweij J, Casali J, Zalcberg A, LeCesne P, Reichard P, et al. 2003. Early efficacy comparison of two doses of imatinib for the treatment of advanced gastrointestinal stromal tumors (GIST): Interim results of a randomized phase III trial from the EORTC-STBSG, ISG and AGITG. *Proc. Am. Soc. Clin. Oncol.* 22:3272 (Abstr.)
91. Heinrich MC, Corless CL, Demetri GD, Blanke CD, von Mehren M, et al. 2003. Kinase mutations and imatinib response in patients with metastatic gastrointestinal stromal tumor. *J. Clin. Oncol.* 21:4342–49
92. Metcalfe DD, Akin C. 2001. Mastocytosis: molecular mechanisms and clinical disease heterogeneity. *Leuk. Res.* 25:577–82
93. Cohen MH, Williams G, Johnson JR, Duan J, Gobburu J, et al. 2002. Approval summary for imatinib mesylate capsules in the treatment of chronic myelogenous leukemia. *Clin. Cancer. Res.* 8:935–42
94. Dagher R, Cohen M, Williams G, Rothmann M, Gobburu J, et al. 2002. Approval summary: imatinib mesylate in the treatment of metastatic and/or unresectable malignant gastrointestinal stromal tumors. *Clin. Cancer Res.* 8:3034–38
95. Druker BJ, Talpaz M, Resta DJ, Peng B, Buchdunger E, et al. 2001. Efficacy and safety of a specific inhibitor of the BCR-ABL tyrosine kinase in chronic myeloid leukemia. *N. Engl. J. Med.* 344:1031–37
96. Trempat P, Villalva C, Laurent G, Armstrong F, Delsol G, et al. 2003. Chronic myeloproliferative disorders with rearrangement of the platelet-derived growth factor alpha receptor: a new clinical target for STI571/Glivec. *Oncogene* 22:5702–06
97. Cunningham D, Humblet Y, Siena S, Khayat D, Bleiberg H, et al. 2003. Cetuximab (C225) alone or in combination with irinotecan (CPT-11) in patients with epidermal growth factor receptor (EGFR)-positive, irinotecan-refractory metastatic colorectal cancer (MCRC). *Proc. Am. Soc. Clin. Oncol.* 22:1012 (Abstr.)
98. Saltz LB, Meropol NJ, Loehrer PJ Sr, Needle MN, Kopit J, Mayer RJ. 2004. Phase II trial of cetuximab in patients with refractory colorectal cancer that expresses the epidermal growth factor receptor. *J. Clin. Oncol.* 22:1201–08
99. Hurwitz H, Fehrenbacher L, Cartwright T, Hainsworth W, Heim W, et al. 2003. Bevacizumab (a monoclonal antibody to vascular endothelial growth factor) prolongs survival in first-line colorectal cancer (CRC): Results of a phase III trial of bevacizumab in combination with bolus IFL (irinotecan, 5-fluorouracil, leucovorin) as first-line therapy in subjects with metastatic CRC. *Proc. Am. Soc. Clin. Oncol.* 22:3646 (Abstr.)
100. Kabbinavar F, Hurwitz HI, Fehrenbacher L, Meropol NJ, Novotny WF, et al. 2003. Phase II, randomized trial comparing bevacizumab plus fluorouracil (FU)/leucovorin (LV) with FU/LV alone in patients with metastatic colorectal cancer. *J. Clin. Oncol.* 21:60–65
101. Slamon DJ, Godolphin W, Jones LA, Holt JA, Wong SG, et al. 1989. Studies of the HER-2/neu proto-oncogene in human breast and ovarian cancer. *Science* 244:707–12
102. Cobleigh MA, Vogel CL, Tripathy D, Robert NJ, Scholl S, et al. 1999. Multinational study of the efficacy and safety of humanized anti-HER2 monoclonal antibody in women who have HER2-overexpressing metastatic breast cancer that has progressed after chemotherapy for metastatic disease. *J. Clin. Oncol.* 17: 2639–48

103. Slamon DJ, Leyland-Jones B, Shak S, Fuchs H, Paton V, et al. 2001. Use of chemotherapy plus a monoclonal antibody against HER2 for metastatic breast cancer that overexpresses HER2. *N. Engl. J. Med.* 344:783–92
104. Kim ES, Vokes EE, Kies MS. 2004. Cetuximab in cancers of the lung and head & neck. *Semin. Oncol.* 31:61–67
105. Salomon DS, Brandt R, Ciardiello F, Normanno N. 1995. Epidermal growth factor-related peptides and their receptors in human malignancies. *Crit. Rev. Oncol. Hematol.* 19:183–232
106. Ullrich A, Coussens L, Hayflick JS, Dull TJ, Gray A, et al. 1984. Human epidermal growth factor receptor cDNA sequence and aberrant expression of the amplified gene in A431 epidermoid carcinoma cells. *Nature* 309:418–25
107. Carpenter G, Cohen S. 1990. Epidermal growth factor. *J. Biol. Chem.* 265:7709–12
108. Schlessinger J. 1988. The epidermal growth factor receptor as a multifunctional allosteric protein. *Biochemistry* 27: 3119–23
109. Yarden Y, Sliwkowski MX. 2001. Untangling the ErbB signalling network. *Nat. Rev. Mol. Cell Biol.* 2:127–37
110. Arteaga CL. 2002. Overview of epidermal growth factor receptor biology and its role as a therapeutic target in human neoplasia. *Semin. Oncol.* 29:3–9
111. Wells A. 1999. EGF receptor. *Int. J. Biochem. Cell Biol.* 31:637–43
112. Yarden Y. 2001. The EGFR family and its ligands in human cancer. signalling mechanisms and therapeutic opportunities. *Eur. J. Cancer* 37 (Suppl. 4):S3–S8
113. Shvartsman SY, Hagan MP, Yacoub A, Dent P, Wiley HS, Lauffenburger DA. 2002. Autocrine loops with positive feedback enable context-dependent cell signaling. *Am. J. Physiol. Cell Physiol.* 282:C545–59
114. Grandis JR, Sok JC. 2004. Signaling through the epidermal growth factor receptor during the development of malignancy. *Pharmacol. Ther.* 102:37–46
115. Gullick WJ. 1991. Prevalence of aberrant expression of the epidermal growth factor receptor in human cancers. *Br. Med. Bull.* 47:87–98
116. Nicholson RI, Gee JM, Harper ME. 2001. EGFR and cancer prognosis. *Eur. J. Cancer* 37 (Suppl. 4):S9–15
117. Cohen MH, Williams GA, Sridhara R, Chen G, Pazdur R. 2003. FDA drug approval summary: gefitinib (ZD1839) (Iressa) tablets. *Oncologist* 8:303–06
118. Kies MS, Harari PM. 2002. Cetuximab (Imclone/Merck/Bristol-Myers Squibb). *Curr. Opin. Investig. Drugs* 3:1092–100
119. Khalil MY, Grandis JR, Shin DM. 2003. Targeting epidermal growth factor receptor: novel therapeutics in the management of cancer. *Expert. Rev. Anticancer Ther.* 3:367–80
120. Slamon DJ, Clark GM, Wong SG, Levin WJ, Ullrich A, McGuire WL. 1987. Human breast cancer: correlation of relapse and survival with amplification of the HER-2/neu oncogene. *Science* 235:177–82
121. Grunwald V, Hidalgo M. 2003. Development of the epidermal growth factor receptor inhibitor OSI-774. *Semin. Oncol.* 30:23–31
122. Ranson M, Mansoor W, Jayson G. 2002. ZD1839 (IRESSA): a selective EGFR-TK inhibitor. *Expert. Rev. Anticancer Ther.* 2: 161–68
123. Wakeling AE, Guy SP, Woodburn JR, Ashton SE, Curry BJ, et al. 2002. ZD1839 (Iressa): an orally active inhibitor of epidermal growth factor signaling with potential for cancer therapy. *Cancer Res.* 62:5749–54
124. Bianco C, Tortora G, Bianco R, Caputo R, Veneziani BM, et al. 2002. Enhancement of antitumor activity of ionizing radiation by combined treatment with the selective epidermal growth factor receptor-tyrosine kinase inhibitor ZD1839 (Iressa). *Clin. Cancer Res.* 8:3250–58

125. Ciardiello F, Caputo R, Bianco R, Damiano V, Pomatico G, et al. 2000. Antitumor effect and potentiation of cytotoxic drugs activity in human cancer cells by ZD-1839 (Iressa), an epidermal growth factor receptor-selective tyrosine kinase inhibitor. *Clin. Cancer Res.* 6:2053–63
126. Normanno N, Campiglio M, De LA, Somenzi G, Maiello M, et al. 2002. Cooperative inhibitory effect of ZD1839 (Iressa) in combination with trastuzumab (Herceptin) on human breast cancer cell growth. *Ann. Oncol.* 13:65–72
127. Fukuoka M, Yano S, Giaccone G, Tamura T, Nakagawa K, et al. 2003. Multi-institutional randomized phase II trial of gefitinib for previously treated patients with advanced non-small-cell lung cancer. *J. Clin. Oncol.* 21:2237–46
128. Sirotnak FM. 2003. Studies with ZD1839 in preclinical models. *Semin. Oncol.* 30: 12–20
129. Perez-Soler R CA, Huberman M. 2004. Final Results from a phase II study of erlotinib (Tarceva) monotherapy in patients with advanced non-small cell lung cancer following failure of platinum-based chemotherapy. *Lung Cancer* 42 (Suppl. 2):246 (Abstr.)
130. Baselga J. 2001. The EGFR as a target for anticancer therapy–focus on cetuximab. *Eur. J. Cancer* 37 (Suppl. 4):S16–22
131. Bergers G, Benjamin LE. 2003. Tumorigenesis and the angiogenic switch. *Nat. Rev. Cancer* 3:401–10
132. Miller KD, Rugo HS, Cobleigh MA. MP-KCLI. 2002. Phase III trial of capecitabine (Xeloda) plus bevacizumab (Avastin) versus capecitabine alone in women with metastatic breast cancer previously treated with an anthracycline and taxane. *Breast Cancer Res. Treat.* 76 (Suppl. 1):37 (Abstr.)
133. Yang JC, Haworth L, Sherry RM, Hwu P, Schwartzentruber DJ, et al. 2003. A randomized trial of bevacizumab, an anti-vascular endothelial growth factor antibody, for metastatic renal cancer. *N. Engl. J. Med.* 349:427–34
134. Giaccone G, Herbst RS, Manegold C, Scagliotti G, Rosel R, et al. 2004. Gefitinib in cominbation with gemcitabine and cisplatin in advanced non-small-cell lung cancer: a phase III trial—INTACT 1. *J. Clin. Oncol.* 22:777–84
135. Herbst RS, Giaccone G, Schiller JH, Natale RB, Miller V, et al. 2004. Gefitinib in combination with paclitaxel and carboplatin in advanced non-small-cell lung cancer: a phase III trial—INTACT 2. *J. Clin. Oncol.* 22:785–94
136. Basel, 26 April 2004. New cancer drug Tarceva—a breakthrough with significant survival benefit in patients with advanced lung cancer. http://www.gene.com/gene/news/press-releases/display.do?method=detail&id=7387
137. Lynch Tomas J, Bell DW, Sordella R, Gurubhagavatula S, Okimoto RA, et al. 2004. Activating mutations in the epidermal growth factor receptor underlying responsiveness of non-small cell lung cancer to gefitinib. *New Engl. J. Med.* 350:2129–39
138. Hirota S, Isozaki K, Moriyama Y, Sashimoto K, Nishida T, et al. 1998. Gain-of-function mutations of c-kit in human gastrointestinal stromal tumors. *Science* 279:577–80

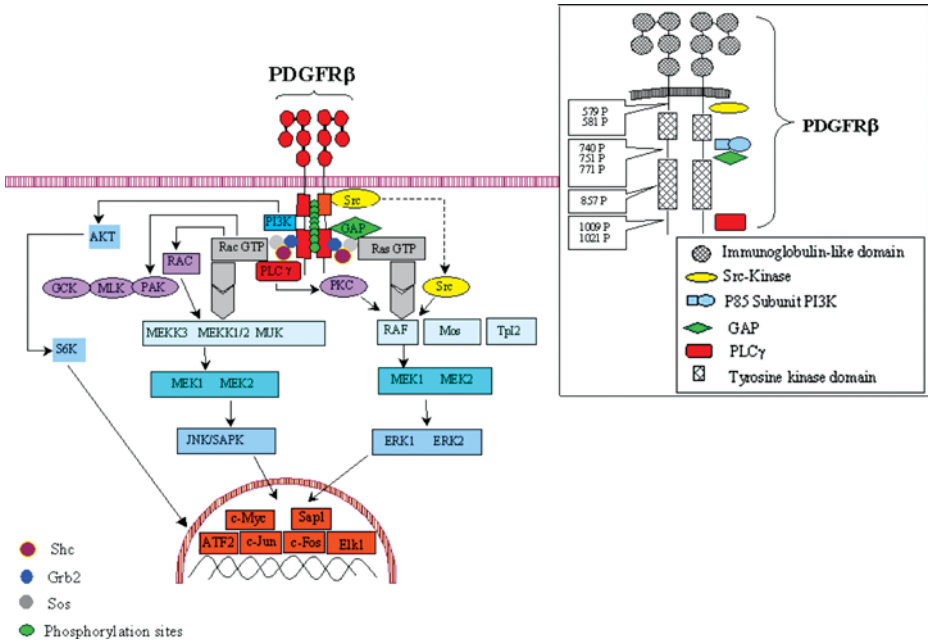

Figure 1 PDGFR signaling Cascade: PDGFR β is used as an example. Binding of PDGF ligand to extracellular receptor domains leads to dimerization of receptor subunits, followed by receptor autophosphorylation creating docking sites for several molecules leading to induction of parallel intracellular signaling pathways. This process mediates pleiotropic biologic effects, including regulation of proliferation, cell cycle progression, apoptosis, survival, and cell migration. Coupling proteins, such as PI3K, PLCγ, Src and others, facilitate GDP-GTP exchange and phosphorylation. Other molecules, such as Grb2, Shc, and Crk, are devoid of enzymatic activity but link the receptor to downstream targets. PI3K activates AKT, which promotes cell survival through effects on transcription factors and inhibition of apoptosis. Rho/Rac are involved in cellular structure, actin organization and chemotaxis. The Ras/MAP kinase pathway is initiated by the adapter molecule Grb2, which forms a complex with Sos. Raf stimulation of the MAPK pathway results in proliferation mediated by nuclear transcription factors. PLCγ phosphorylates PKC, which is a Ras-independent activator of the MAPK pathway. Recruitment of src tyrosine kinase activates various cascades including the transcription factor c-myc. Not depicted in the figure is the JAK/STAT pathway, in which STATs translocate from the membrane to the nucleus and directly activate cytokine-responsive genes. Insert: Structure of the PDGFR β. The extracellular region of PDGFRβ has five immunoglobulin chain-like sites. The intracellular sites most frequently autophosphorylated are shown by the numbers.

Annual Review of Pharmacology and Toxicology
Volume 45, 2005

CONTENTS