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Review Article

Tyrosine kinase targets in drug discovery

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CONTENTS

Abstract

Protein tyrosine kinases are central to the signal transduction machinery of healthy cells and their deregulation plays a causal role in many diseases, particularly in cancer. Inhibition of the altered activity of tyrosine kinases and restoration of physiological balance is being explored as a means of developing new therapeutic interventions. A number of small-molecule inhibitors and monoclonal antibodies, among other approaches, are currently being evaluated in cancer chemotherapy. The preclinical and clinical development of these agents, issues relevant to their progress and possible novel tyrosine kinase targets are discussed.

Introduction

Virtually all mammalian cells carry a similar molecular machinery which provides an integrated cell circuit regulating to their proliferation, differentiation and death. Protein phosphorylation regulates the function of this cellular machinery by serving as a major mechanism for transmembrane and intracellular signaling. Phosphorylation is, in turn, tightly controlled by protein kinases and protein phosphatases. Protein kinases are enzymes catalyzing the transfer of the terminal (γ) phoshoryl group of ATP to specific amino acid residues, thus altering protein structure and ultimately affecting ligand binding and catalytic activity. The rate of this reversible, covalent modification is regulated according to physiological needs and effects are amplified and relayed through a signal transduction cascade. Based on the specificity of their catalytic activity, protein kinases can be subdivided into tyrosine- or serine/threonine-specific. In addition, some possess dual specificity for both tyrosine and serine/threonine and a few members of the phosphatidylinositol family (a family of lipid kinases) also exhibit protein-serine/threonine kinase activity. The evolutionarily conserved families of tyrosine kinases are extensively used components of the cell's signal transduction machinery and can be found as membrane-spanning receptors (Fig. 1) or in cytoplasmic forms (Fig. 2). The protein kinase complement of the human genome (kinome) consists of 30 tyrosine kinase families containing about 90 distinct protein tyrosine kinases (PTKs), of which 58 members are receptor tyrosine kinases (RTKs) (1).

In the same manner, tyrosine kinases are central to physiological homeostasis. Their deregulation by mutations/rearrangements or overexpression plays a causal role in human disease, affording the possibility of developing agonists and antagonists of these enzymes for therapeutic use. This is particularly true in cancer therapy since tyrosine kinases are in many ways directly or indirectly linked to the etiology and progression of the disease. Under the modern chemotherapy principle that innovative molecular therapies should be targeted specifically to the molecular pathology of cancer, tyrosine kinases provide favorable targets, enabling stepwise concept validation at biochemical, cellular and *in vivo* levels (2, 3). Currently, more than 20 different tyrosine kinase targets are under evaluation in drug discovery projects in oncology (3). This is, in part, due to the remarkable success of the small-molecule tyrosine kinase inhibitor, imatinib mesylate (Glivec®, Gleevec™, STI-571; developed by Novartis) in the treatment of chronic myelogenous leukemia (CML), gastrointestinal stromal cell tumors (GIST) and metastatic dermatofibrosarcoma protuberans, conditions that are dependent on the expression and activity of the p210bcr-abl and c-kit kinases and PDGFβ, respectively (4-6). In addition, demonstration of inhibition of the target in the clinic (*i.e.*, clinical proof of concept) has been achieved with several antibodies (*e.g.*, trastuzumab and cetuximab) and low-molecular-weight tyrosine kinase

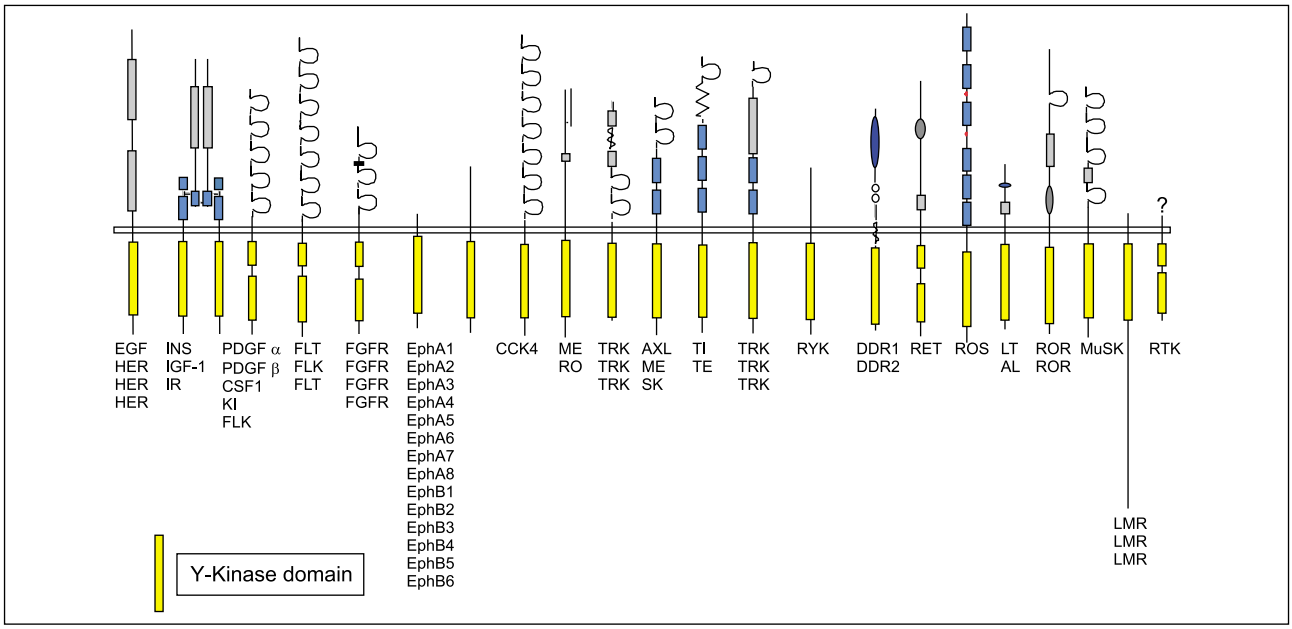

Fig. 1. Schematic representation of the different families of the receptor tyrosine kinases.

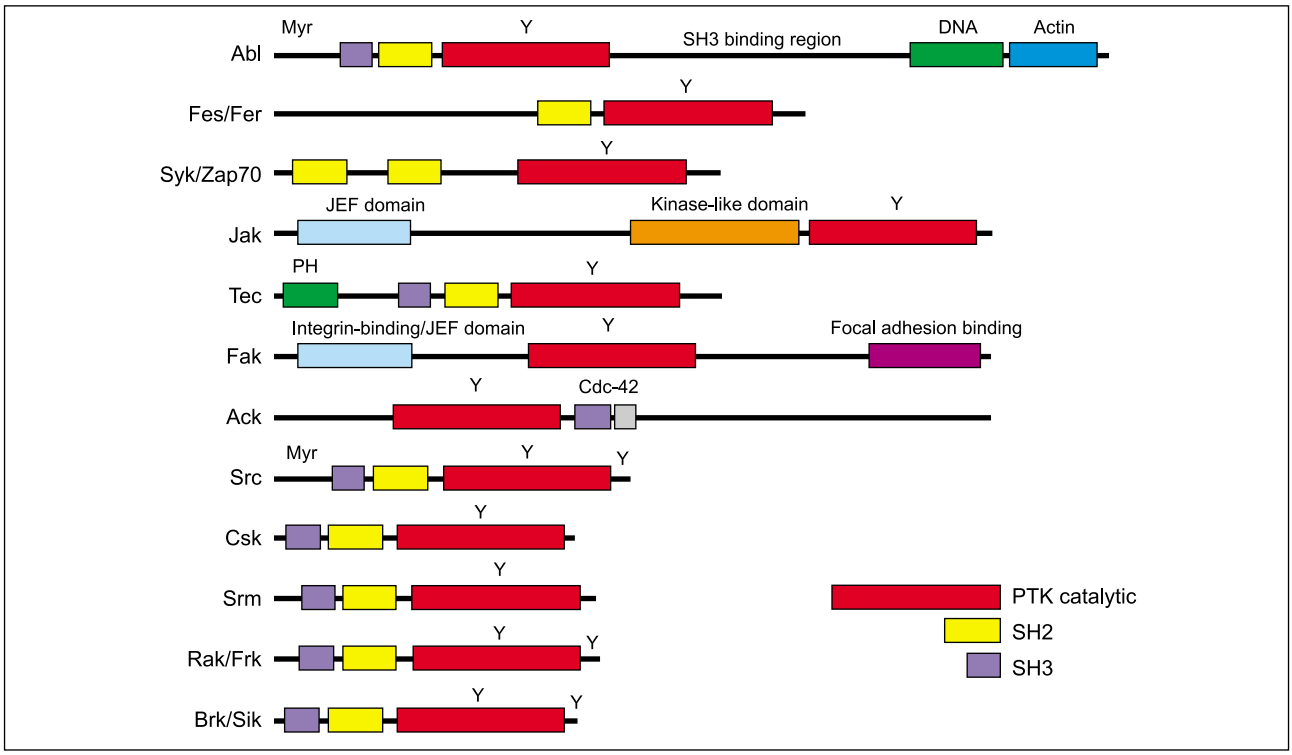

Fig. 2. Schematic representation of the different families of the nonreceptor tyrosine kinases.

inhibitors (*e.g.*, imatinib mesylate, gefitinib, erlotinib hydrochloride, PKI-116, PTK-787/ZK-222584, SU-5416 and ZD-6474). These results have largely overridden concerns which previously argued against protein kinases as suitable targets (7) which include high intracellular ATP concentrations *versus* ATP site directed inhibitors, a common catalytic mechanism across the many families of kinases, structural similarities of other features of the kinase enzyme active center and the importance of kinase activities in many physiological processes. At the

same time, rational, structure-based drug design has been aided by the construction of credible pharmacophore models and the elucidation of the crystal structure of many PTKs, thus providing architectural detail of the active site targets. Further technological advantages provided by combinatorial chemistry and high-throughput screening have also assisted novel drug design (8-11).

Tyrosine kinase signaling

RTKs are transmembrane proteins with a cytoplasmic domain possessing intrinsic catalytic activity that is activated upon ligand binding. Once activated, receptor dimerization (or oligomerization) juxtaposes the two catalytic domains allowing mutual transphosphorylation of residues in the activation loop of the catalytic domain. This leads to enzyme activation and autophosphorylation of tyrosine residues outside the catalytic domain. These phosphorylated tyrosine residues serve as docking sites for phosphotyrosine binding domains (*e.g.*, Src homology 2 and 3 [SH2 and SH3] and phosphotyrosine binding [PTB] domains) found in a number of intracellular signaling proteins (*e.g.*, Shc, Grb2, Src, Cbl, phospholipase Cg and phoshoinositol-3' [PI-3'] kinase). Assembly of activated complexes at the membrane initiates several cascades which are the key to downstream signaling and biological response (12-16). Formation of homo- or heterodimers is also possible as in the case of EGFR or Tie receptors (17, 18). Receptors lacking catalytic activity can be coupled to nonreceptor PTKs via noncovalent association with the cytoplasmic domain of a receptor subunit, thus forming "binary" receptors (19).

The most important downstream signaling cascades activated by RTKs include the Ras-extracellular regulated kinase (ERK)-mitogen activated (MAP) kinase pathway, the PI-3'kinase-AKT and the JAK/STAT pathway. PTKs provide communication signals that link all these pathways ultimately leading to regulation of gene transcription. For example, SH2/PTB domains are also present in so-called adaptor proteins, such as Grb2, that bind to and thereby bring effector enzymes to the plasma membrane. Grb2 complexes with the Cdc25-related Sos protein which, when recruited to the plasma membrane to an activated RTK, bring the Sos catalytic domain into proximity with Ras and stimulate GTP exchange, thus activating Ras (20). This, in turn, leads to interactions with a series of other effector proteins including the Raf protein-serine kinase and PI-3' kinase, initiating downstream signaling along the RTK-Ras-MAP kinase pathway leading to transcriptional control (21). Additional cascades may also be utilized. For example, the InsR utilizes the adenylyl cyclase signaling system which, in turn, activates cAMP-dependent serine-threonine specific protein kinases (22).

Nonreceptor tyrosine kinases participate in response to extracellular signals by physically associating with transmembrane receptors, such as hormone, cytokine and growth factor receptors. They are then activated when these receptors are bound by extracellular ligands or cell adhesion components at particular phases of the cell cycle. For example, Scr is associated and activated by PDGFR and Scr-like tyrosine kinases have been shown to associate with the CD4, CD8 antigens and the T-cell antigen receptor complex in T-cells (23-25). ErbB also activates STAT transcription factors via Scr tyrosine kinases (25). Similarly, ligand binding to a cytokine receptor activates the appropriate JAK family member PTKs, which first phosphorylate the receptor subunit and then specific STAT transcription factors. The signal is finally transmitted when STAT factors dimerize, migrate to the nucleus and activate transcription (26).

Generally, PTKs facilitate transduction of a diverse array of extracellular signals via repeated use of common cytoplasmic signaling pathways. It is extremely intriguing and an area of extensive investigation as to how reiterative deployment of the same signaling cascade engenders context specific and appropriate cellular responses. Certain common aspects in the function of PTKs are becoming apparent. On the whole, specificity of signal transduction is afforded by the fact that the cascade of phosphorylation steps is sequence-dependent, assembly of signaling complexes is necessary and signaling can be initiated and propagated using the principle of induced protein proximity (15).

Additional interesting properties in the functions of specific families of PTKs are also becoming better understood. In the case of Ephs and their ligands, for example, their interactions at sites of cell-to-cell contact initiate unique bidirectional signaling cascades where the information is transduced in both the receptor and the ligand-expressing cells (27). For some PTKs, evidence has suggested that dimerization *per se* may not be sufficient for activation and that there is an additional requirement for the orientation of the two dimer subunits to be arranged such that the catalytic domains are correctly juxtaposed, as for example with Src (28). The coupling of a given receptor to specific intracellular signaling proteins is modulated by the dimerization partner and may originate from differential receptor phosphorylation, as has been shown for EGFR and ErbB2 (29). In addition, a heterodimer can acquire novel signaling properties that are distinct from the activity of the individual receptors (29). Negative receptor signaling resulting in attenuation of the ligand-induced signal and subsequent modulation of the level of cell stimulation is also important for receptor tyrosine kinases activity and involves coordinated actions of ubiquitin ligases (*i.e.*, Cbl), adaptor proteins (*i.e.*, Grb2 and CIN85), inhibitory molecules (*i.e.*, sprouty), cytoplasmic kinases (*i.e.*, activated Cdc42-associated kinase) and phosphoinositol metabolites (30). Regions with autoinhibitory function within RTKs also exist (as in Tie2) and in some cases, the juxtamembrane region has been implicated in autoinhibition (*e.g.*, PDGFR, Kit, Eph) and autophosphorylation of these residues relieves the inhibitory conformation enabling full kinase activation and, at the same time, creates binding sites (31). Finally, a fundamental outcome of inhibitory regulation is

elevation of the threshold for receptor activation and subsequent initiation of the required downstream signal transduction cascade, thus offering the possibility of distinguishing between different signals and initiation of the appropriate cascade. This also implies that a threshold of signal intensity is necessary for full receptor activation and recruitment of effector proteins before receptor internalization (16, 32).

Tyrosine kinase signaling in disease

In normal cells, activated RTKs are rapidly internalized away from the cell surface and are subject to modifications that inhibit their enzymatic activity. This ensures that activation of signal cascades are only transient and the cell returns to its non-stimulated state in a timely fashion. However, a variety of structural alterations ranging from single amino acid substitutions to large deletions, or deregulation of inhibitory signals and autocontrol mechanisms, can lock kinases into the activated form in which the kinase domain is always active. A number of diseases have been shown to be due to mutations that activate or lead to misexpression/overexpression of PTKs (33, 34). During molecular characterization of malignancies, approximately half of all known PTKs such as EGF, ErbB2, Ret, Kit, Scr, Abl, IGF1R, VEGF1/2/3, FGFR1/2/3, *etc.*, have been found in either mutated or overexpressed forms including sporadic cases (33). The general principles for oncogenic transformation by PTKs include retroviral transduction of a protooncogene corresponding to a PTK concomitant with deregulating structural changes, a common mechanism in rodents and chickens (*e.g.*, *c-scr*); genomic rearrangements such as chromosomal translocations can result in oncogenic fusion proteins that include (minimally) a PTK catalytic domain and an unrelated protein that provides a dimerization function (*e.g.*, Abl, PDGFRb); a gain of function mutations or small deletions (*e.g.*, *ret*, *c-kit*); and PTK overexpression resulting from gene amplification (*e.g.*, *c-erbB1*,*2*,*3*) (33).

Mutations in PTKs are also involved in diseases other than cancer. Mutational inactivation of nonreceptor tyrosine kinases is observed in several immunodeficiencies. Inactivation of both copies of JAK3 causes severe combined immunodeficiency (35, 36). Mutation in the Bruton tyrosine kinase (BTK, also known as BPK or ATK), a member of the scr family and a key regulator of B-cell maturation, causes X-linked Agammaglobulinemia. BTK signaling is mediated through interactions with FYN, LYN and HCK nonreceptor protein kinases, which are activated upon stimulation of B- and T-cell receptors (37, 38). The physiological role of PTK in CNS signaling also suggests that deregulation of these proteins might also be involved in related disorders (39). This is supported by the recent observation that neuregulin-1 and erbB4 immunoreactivity is associated with neuritic plaques in the Alzheimer's disease brain and in a transgenic model of Alzheimer's disease (40).

Irrespective of the underlying genetic alteration, the outcome *i.e.*, altered, aberrant or inappropriate receptor presence, gives rise to respective disease phenotypes (*e.g.*, cancer). This is not however, maintained only by receptor deregulation but also in the context of the whole cell circuit and intra-/intercellular communications, *i.e.*, a multitude of paracrine and autocrine communications. Growth factors (*e.g.*, EGF, VEGF PDGF), their receptors and growth inhibiting factors are frequently overexpressed in cancers and their coexpression (as opposed to individual factor or receptor expression) is often associated with tumor cell proliferation and other tumor parameters such as angiogenesis and metastasis (41, 42). Cancer in itself can be defined as a pathology of crosstalk among signaling proteins but, as mentioned above, other conditions also share similar molecular pathologies. On the other hand, a number of diseases are due to insufficient PTK signaling, such as non-insulin-dependent diabetes and peripheral neuropathies, and in such cases methods to enhance signaling could serve as viable therapies (15). Some insulin mimetic compounds are being developed (43, 44). This is also a very attractive possibility for other angiogenesis-related conditions, including certain cardiovascular diseases where stimulation of angiogenesis might be required rather than inhibition.

Tyrosine kinase inhibitors

During tyrosine kinase signal transduction, each substrate in the phosphotransfer reaction, the tyrosine hydroxy group and ATP, represent reasonable pharmacological starting points for the design of substrate analogues or ATP competitive inhibitors. Drug design strategies have aimed at the development of selective components that target either the extracellular ligand binding domain, the intracellular tyrosine kinase or the substrate binding region. Despite initial skepticism, among the small-molecule inhibitors under development, a great number of ATP site directed inhibitors of PTKs have yielded a variety of lead structures (*e.g.*, quinazolines, pyrrolo-pyrimidines, phenylamino-pyrimidines, substituted indolin-2-ones and natural products such as staurosporine) (45-48). The majority of compounds in development target members of the EGFR and VEGFR families and with the exception of Abl, they all target RTKs. Because anticancer treatment is envisioned as a chronic therapy, orally active, small-molecule inhibitors have been preferred. The available clinical data so far have indicated that treatment with EGFR targeting agents, whether kinase inhibitors or blocking antibodies, in single agent regimens, is primarily cytostatic rather than cytoreductive. Some cytoreductive action is however also inferred since few partial responses were observed with the small-molecule inhibitor gefitinib and the monoclonal antibody trastuzumab. Compounds currently in clinical development are shown in Table I and some of these are discussed below.

Table I: Tyrosine kinase inhibitors in clinical development.

Target	Compound	Source	Phase
Small molecule kinase inhibitors			
Bcr-Abl, c-Kit, PDGF	Imatinib mesylate (Glivec®, Gleevec™)	Novartis	L-2001
EGFR	Gefitinib (Iressa™)	AstraZeneca	L-2002
EGFR	Erlotinib hydrochloride (Tarceva™)	Roche/Genentech/OSI	III
EGFR, ErbB2, ErbB3/4	CI-1033 (irreversible inhibitor)	Pfizer	II
EGFR, ErbB2	EKB-569 (irreversible inhibitor)	Wyeth-Ayerst	II
EGFR, ErbB2	GW-2016	GlaxoSmithKline	II
VEGFR	PTK-787/ZK-22584	Novartis/Schering	II/III
VEGFR (EGFR)	ZD-6474	AstraZeneca	II
PDGFR, VEGFR, FGFR	SU-6668	Pharmacia/Sugen	I/II
VEGFR, PDGF, c-kit, Flt-3	SU-11248	Pharmacia/Sugen	I/II
VEGFR, FGFR	CP-547632	Pfizer	I/II
VEGFR, FGFR, PDGFR (Flt-4)	CHR-200131	Chiron	I
VEGFR, PDGFR, Flt-3	CEP-7055	Cephalon	I
VEGFR, PDGFR	AG-13736	Pfizer/Agouron	I
VEGFR, c-Kit	KRN-633	Kirin	I
Flt-3, PKC (PDGFR, c-kit)	PKC-412	Novartis	II
Flt-3	CT-53518	Millennium	I
Flt-3	CEP-701	Cephalon	II
Monocloncal antibodies			
ErbB2	Trastuzumab (Herceptin®)	Genentech	L-1998
ErbB2	MDX-210	Medarex	I
ErbB2	2C4	Genentech	I
EGFR	IMC-1C11	ImClone Systems	I
EGFR	Cetuximab (Erbitux™)	ImClone Systems	III
EGFR	ABX-EGF	Abgenix	II
EGFR	EMD-72000	Merck KgaA	I
EGFR	RH3	York Medical	II
EGFR	MDX-447	Medarex/Merck KgaA	I
ErbB2	BsAB 2B-1	Chiron	IB/II
VEGFR2	HuMV833	EORTC	I
PDGFβ	CDP-860	Celltech	I/II
Other approaches			
ErbB2	APC8024 (vaccine)	Dendreon	I
EGFR	DAB389EGF (hDT-hEGF fusion protein)	Seragen	II
VGFR1 mRNA	PRI.4610 (nuclease-stabilized hairpin ribozyme)	Ribozyme Pharm.	I/II
IGF 1R mRNA	INX-4437 (antisense oligonucleotide)	INEX	I
ErbB2	17-AAG (geldanamycin derivative inhibits Hsp90)	Kosan	I

Epidermal growth factor receptor inhibitors

The EGF family of receptor tyrosine kinases, including HER-1 (c-ErbB1/EGFR), HER-2 (c-ErbB2/neu), HER-3 (c-ErbB3) and HER-4 (c-ErbB4), are overexpressed and strongly implicated in the development and progression of numerous human tumors including breast, lung, gliomas, bladder, *etc.* (49-54). These receptors are bound by 4 types of ligands: EGF, amphiregulin (AR) and transforming growth factor alpha (TGFα) bind ErbB1; betacellulin (BTC), heparin binding EGF (HB-EGF) and epiregulin (EPR) bind ErbB1 and ErbB4; neuregulin (NRG)-1 and NFG-2 bind ErbB3; NRG-3 and NRG-4 bind ErbB4 (17). No direct ligand for ErbB2 has yet been discovered and it appears to act as a preferential coreceptor increasing the affinity of ligand binding to the dimeric receptor complex (55, 56). Overexpression of ErbB2 is observed in a significant proportion of breast and ovarian tumors where it is associated with poor prognosis, as well as other tumors including gastric and colorectal carcinomas (57, 58). Furthermore, many breast tumors display autocrine activation of ErbB1 due to expression of one or more ErbB1 ligands (59). EGFR mutations can also give rise to truncated forms of these receptors (*e.g.*, EGFRvIII) which lack extracellular domains and the ability to bind their ligands but nevertheless display constitutive kinase activity (60, 61). In addition to antitumor cell effects, EGFR-specific inhibitors are also expected to display antiangiogenic effects since blockade of EGFR signaling has also been shown to lead to endothelial cell apoptosis

(62). The clear association of EGFR with the etiology of cancer has prompted extensive drug discovery programs by various companies which have so far yielded several antibodies and low-molecular-weight, ATP competitive EGFR tyrosine kinase inhibitors in clinical development. The observation that EGFR blockade renders tumor cells more sensitive to apoptosis as compared to normal cells also strengthens the case for use of such inhibitors (63).

Currently, the most advanced small-molecule inhibitor in clinical development is gefitinib (Iressa™, ZD-1839; AstraZeneca), which was first approved in Japan last year and was recently granted approval by the FDA. *In vitro*, gefitinib blocks EGFR-dependent proliferation and autophosphorylation resulting in inhibition of downstream targets (*e.g.*, MAPK/Akt) and apoptosis, as well as attenuation of angiogenic growth factor production by tumor cells (64-67). The fact that it also suppresses ErbB2 signaling in ErbB2 overexpressing cells even though it is selective for EGFR indicates the important role of ErbB2 in signaling via EGFR/ErbB2 heterodimerization (68). This additionally demonstrates that by exploiting cross-talk between EGFR and ErbB2, selective inhibitors may be effective in a broader spectrum of tumors (69). Preclinical evaluation of gefitinib demonstrated potent activity in a series of xenografts, as well as additive/synergistic effects with other chemotherapeutics and radiation (70-73). Phase I and II trials in non-small cell lung cancer (NSCLC) and other solid tumors were completed with positive feedback on tolerability and pharmacodynamic effects (74, 75). The most common adverse effects included skin rash, diarrhea, fatigue and minor events of liver toxicity (*i.e.*, elevated transaminases) (74, 75). In single-agent treatment regimens, partial/minor responses and stable disease were reported for several tumor types and various clinical trials in combination with other modalities (including trastuzumab) are ongoing (http://www.clinicaltrials.gov). In June 2002, gefitinib was granted approval in Japan as a single agent for the treatment of refractory NSCLC. However, since then, serious lung injuries such as interstitial pneumonia were reported in 125 patients, including at least 39 deaths. However, an increase in incidence of such syndromes was not observed in the 2 large-scale phase III trials (INTACT) in patients with NSCLC although no clear benefit of gefitinib in combination with cytotoxics was demonstrated (3). On May 5, 2003, the FDA granted accelerated approval of gefitinib as a single-agent treatment for patients with advanced NSCLC whose cancer has continued to progress despite treatment with platinum-based and docetaxel chemotherapy.

Erlotinib hydrochloride (Tarceva™, OSI-774; Roche/Genentech/OSI), in phase III development, is another orally active inhibitor of EGFR tyrosine kinase activity that induces cell cycle arrest and apoptosis by long-lasting reduction in EGFR autophosphorylation in various human tumor xenografts (76, 77). In combination with cisplatin, additive antitumor activity and enhanced apoptosis through suppression of Akt have been demonstrated in experimental systems (78). In phase I/II clinical trials, side effects similar to those reported with gefitinib have been observed. Partial responses for NSCLC and head and neck cancer have been reported in a number of clinical trials currently under way with the drug as a single agent or in combination with other drugs (http://www.clinicaltrials.gov). Another small molecule, GW-2016 (GlaxoSmithKline) has equipotent low nanomolar activity against both EGFR and ErbB2 and after successful preclinical evaluation and is currently in phase II clinical trials (79, 80).

Research activities aimed at the rational design of irreversible EGFR inhibitors which covalently bind to the ATP binding domain of the receptor successfully led to the identification of 2 interesting candidates, namely, CI-1033 (PD-183805; Pfizer) and EKB-569 (Wyeth-Ayerst) (81, 82), both of which are in phase II development. CI-1033, a pan-erbB tyrosine kinase inhibitor, is active against all 4 members of the EGFR tyrosine kinase family. *In vitro* studies in human cancer cell lines indicate that CI-1033 results in prompt, potent and sustained inhibition of tyrosine kinase activity (82). CI-1033 has produced synergistic effects in combination studies with cisplatin, gemcitabine and radiotherapy (83, 84). Some *in vitro* combination studies have indicated an additional mechanism of action. Studies combining the agent with the topoisomerase I inhibitors revealed that the antitumor effects of CI-1033 are not solely related to EGFR inhibition. CI-1033 also binds to the breast cancer resistance binding protein (BCRP), a recently identified ATP binding cassette transporter, thus preventing drug efflux leading to CI-1033 accumulation (85). Skin rash, diarrhea, nausea, emesis and thrombocytopenia were reported as adverse effects in phase I trials (86).

The irreversible inhibitor, EKB-569, has also demonstrated potent inhibition of EGFR and significant *in vivo* antitumor activity in experimental systems (87). One of the most interesting aspects of this compound is the demonstration of synergistic effects in the prevention of polyp formation in a murine model of human familial adenomatous polyposis when given alone or in combination with the COX-1/COX-2 inhibitor, sulindac (88). Evidence that prostaglandin E_2 (PGE_2), a product of COX-1/COX-2 action, rapidly phosphorylates EGFR and triggers the extracellular signal-regulated kinase 2 (ERK2) mitogenic signaling pathway in normal gastric epithelial (RGM1) and colon cancer (Caco-2, LoVo and HT-29) cell lines, provides an explanation for this action of EKB-569 (89). Corresponding combination trials of EKB-569 with COX-2 inhibitors have been initiated.

Relatively recent efforts in tyrosine kinase targeting have focused on ErbB2 selective inhibitors. Such compounds which have already demonstrated significant *in vitro* and *in vivo* inhibitory activities, include Compound 820 (Wyeth-Ayerst), CP-654577 (Pfizer) and TAK-165 (Takeda) (3). For the latter, an extremely high selectivity as compared to EGFR of over 3000-fold has been reported.

Small-molecule inhibitors of other protein tyrosine kinases

The absolute requirement of angiogenesis in all steps of tumor formation and the specificity of expression of the VEGFRs on the vascular endothelium strongly justifies the use of VEGFR inhibitors in the therapy of cancer via inhibition of angiogenesis. It is thought that inhibition of VEGF-induced angiogenic signals will selectively target tumor-associated vessels, since cell division of endothelial cells in the normal vasculature is a rare event. In addition, endothelial cells are believed to be more genetically stable than epithelial cells and therefore less prone to develop resistance mechanisms (90). Antiangiogenic therapy is expected to be safer and better tolerated in cancer patients as compared to cytotoxic therapy, although it might interfere with physiological angiogenesis and therefore restrict patient populations. VEGFR1 and VEGFR2 paracrine loops triggered by the expression of their respective ligands and feeding the angiogenic network in support of the expanding tumor mass have been described for numerous malignancies, such as gliomas, breast and colon tumors (41, 42, 91, 92). Beneficial indirect effects of VEGFR inhibition arise from the fact that VEGF itself acts as a vascular permeability factor and therefore may play a key role in ascitic fluid formation and edema associated with certain types of cancer (93). Also, VEGFR inhibition may restore immune responses against VEGF-secreting tumors, since VEGF itself interferes with maturation of dendritic cells (94). Recent research efforts have also concentrated on inhibition of receptor tyrosine kinase activity of the other member of the VEGFR family, namely VEGFR3 (flt-3), which is primarily expressed by immature hematopoietic cells and important for the normal development of stem cells and the immune system. VEGFR3 has been implicated in acute myeloid leukemia (AML), where two types of activating mutations for VEGFR3 have been described (95-97). One type involves internal tandem duplications within the juxtamembrane domain and occurs in approximately 20-25% of AML patients and the other type consists of point mutations within the “activation loop” involving Asp835 and is found in approximately 7% of patients (98-100). In general, most of the split kinase domain tyrosine kinase inhibitors are in an earlier stage of clinical development as compared to the EGFR family inhibitors and some of these are discussed below.

PTK-787/ZK-222584 (vatalanib succinate; Novartis/Schering) is a potent, selective, VEGFR2 tyrosine kinase inhibitor that also has activity against VEGFR1, PDGFRb, c-Kit and c-Fms. PTK-787/ZK-222584 has demonstrated inhibitory activity in a variety of *in vitro* and *in vivo* angiogenesis assays (101-104). Treatment with the drug has also resulted in antitumor and antimetastatic effects in various tumor xenografts, including HT-29 colon and PC-3 and DU-145 prostate carcinomas (101). VEGF-mediated effects, such as formation of ascites and pleural effusate, were also suppressed during treatment with PTK-787/ZK-222584 in ovarian and lung carcinoma mouse models (105, 106). Phase I clinical trials showed responses and stable disease in different types of advanced tumors (*e.g.*, colorectal, renal, glioma). Reduction in tumor blood flow index and vascular permeability was demonstrated by MRI (3, 107) and an elevation in plasma VEGF was detected, consistent with drug-induced antiangiogenic effects producing tumor hypoxia. PTK-787/ZK-222584 is now proceeding into large phase II/III clinical trials as a single agent or in combination with conventional anticancer therapy.

ZD-6474 (AstraZeneca), presently in phase II development, is a potent VEGFR2 inhibitor, with weaker activity against EGFR and FGFR kinases and, as recently reported, also inhibits transformed Ret kinase (108, 109). Antiangiogenic effects and antitumor activity were observed in experimental models and led to further clinical investigations (110-112). In these studies a long half-life (120 h), linear pharmacokinetics and adverse reactions, including skin rash (interestingly similar to that observed with EGFR inhibitors), were reported in patients with various solid tumors (113). The agent is in phase II clinical trials.

SU-6668 (Pharmacia/Sugen) and SU-11248 (Pharmacia/Sugen), both in phase I/II development, are modified, improved compounds based on the inodolin-2-one lead structure SU-5416. SU-6668 potently inhibits PDGFRb but also blocks VEGFR as well as FGFR (114, 115), while SU-11248 inhibits PDGFRb, c-Kit and Flt-3 phosphorylation (116, 117). Treatment with either compound has resulted in tumor regression mediated by antiangiogenic actions in experimental systems (115-117). Currently, it is unclear whether SU-6668 is being further developed since it did not appear to reach pharmacoactive systemic levels in patients (116). SU-11248 is being investigated in patients with imatinib-refractory GIST (involving c-Kit) and AML (involving Flt-3).

The FGFR family is involved in the regulation of many physiological cell functions and there is strong evidence that deregulated FGF/FGFR signaling contributes to tumorigenesis (overexpression of receptors, stimulation of angiogenesis, inhibition of apoptosis and promotion of resistance to chemotherapy and radiation) (118, 119). Preclinical studies using FGFR targeted inhibition have provided encouraging results. Point mutations in FGFR3 have been identified in human myeloma and in FGFR2 and FGFR3 in bladder, cervical, gastric and colorectal carcinomas (120, 121). However, proof of concept for the efficacy of such agents has yet to be established (122). Similarly, the PDGFR family is widely expressed in normal and malignant cells and the receptor-ligand autocrine loops have been demonstrated to be important in various malignancies, including glioblastomas, melanomas, ovarian, pancreatic, stomach, lung and prostate cancers (123, 124). Currently, however, there are no selective PDGFR or FGFR kinase inhibitors in development. Many VEGFR inhibitors also target VEGFR2/PDGFR (*e.g.*, SU-6668, SU-11248, AG-13736, CHR-200131) or VEGFR2/FGFR (*e.g.*, CP-547632, CHR-200131) or combined Bcr-Abl/Kit/PDGFR (*e.g.*, imatinib mesylate).

The clinical success of imatinib has also encouraged initiation of research programs involving c-Kit/SCFR. Recently, more than 30 germline loss of function mutations have been identified in c-Kit and are associated with cancers such as AML, small cell lung carcinoma, gliomas, testicular cancer and GIST (125, 126). Potent activity against Kit kinase has been described for many EGFR and VEGFR inhibitors, including PTK-787/ZK-222584, PKI-116, PKC-412, SU-6668, SU-11248 and KRN-633 (127). Combination, clinical trials with SU-11248 have been initiated.

A number of other compounds that were not initially developed as specific tyrosine kinase inhibitors have been demonstrated to have significant antityrosine kinase activity in experimental systems. These include PKC-412 (Novartis), a staurosporine derivative originally identified as an inhibitor of protein kinase C (PKC) and subsequently shown to inhibit VEGF-R2, PDGF, Flt-3 and c-kit that may also be beneficial in hypereosinophilic syndromes (128, 129), and CEP-701 (Cephalon), a potent FLT3 and Pan-Trk tyrosine kinase inhibitor (130-132). Both of these compounds are in phase II development. Other new molecules have been reported in recent meetings and include CHR-200131 (Chiron), a VEGFR1/2/3, FGFR and PDGFR kinase inhibitor; CP-547632 (Pfizer), a VEGFR and FGFR kinase inhibitor; AG-13736 (Pfizer/Agouron), a combined VEGFR/PDGFR kinase inhibitor; CEP-7055 (Cephalon), a VEGFR, FGFR kinase inhibitor; and KRN-633 (Kirin), a VEGFR and c-Kit kinase inhibitor (3, 133). CT-53518 (Millennium) has been reported to inhibit Flt-3, PDGFR and c-Kit kinase (134). All of thse newer compounds are in phase I development.

Bcr-Abl tyrosine kinase inhibition

c-Abl protein in itself is thought to be involved in DNA damage-induced apoptosis and to function in the cytoplasm, where it mediates PDGF-induced motility responses and cell adhesion (135, 136). The Philadelphia chromosome, created by the (9;22) reciprocal translocation of the PTK c-Abl to the breakpoint cluster region (bcr) on the long arm of chromosome 22, provided the first example of a consistent chromosomal abnormality associated with a specific type of leukemia (137). Three different versions of this translocation exist, giving rise to a fusion protein with upregulated tyrosine kinase activity. These translocations are found in the majority of patients with CML and in a significant fraction of patients with acute lymphocytic leukemia (ALL). Activation of the Bcr-Abl kinase domain by formation of homooligomeric complexes which allow transphosphorylation, in part, also controls cytoplasmic retention of the protein in transformed cells, thus preventing physiologically driven apoptosis mechanisms (135). The transforming effect of Bcr-Abl is mediated by common RTK-activated pathways including, Ras-Raf-ERK, JAK-STAT and PI(3)K (138).

Exploitation of the clear etiology of Bcr-Abl in CML led to the development of the “model” molecular targeting drug, imatinib. Imatinib is the result of the optimization process of phenylamino-pyrimidine lead scaffold that was originally identified during random screening for PKC inhibitors (139). The compound potently and selectively inhibits various activated Abl fusion proteins in addition to inhibiting signaling from ligand-activated PDGF, c-Kit autophosphorylation and MAPK activation (140-142). Imatinib prevented cell growth of various Bcr-Abl transformed leukemic cells and induced apoptosis in a manner dependent on Stat-5-induced upregulation of the Bcl-2-like Bcl-xL (143, 144). This was also seen in fresh leukemic cells from Ph-positive CML and ALL patients but not from negative ALL and AML primary blast cells (145-147). Following a series of successful clinical trial lasting less than 3 years, the drug was approved in May, 2001 by the FDA for the initial treatment of end-stage CML and, over the following year, also for the treatment of metastatic or unresectable malignant gastrointestinal tumors and treatment of newly diagnosed Ph positive CML (148-152, (http://www.fda.gov/cder/cancer/druglistframe.htm). However, issues with drug resistance in the blast crisis stage of the disease have emerged. Several mechanisms of resistance have been identified from *in vitro* studies with Bcr-Abl-positive cell lines. Mechanisms include amplification or overexpression of Bcr-Abl or an increased expression of P-glycoprotein. (153-155). In a mouse model, the binding of imatinib to acidic α_1 glycoprotein (AAG) has been proposed to be involved in the development of resistance (156). This however, has not been demonstrated in the clinic (157). Studies with clinical samples from resistant patients have shown that point mutations in the kinase domain of Bcr-Abl play a role in the development of resistance to imatinib (158-159). Approximately half of the relapsed patients display an amplification of bcr-abl and a number of point mutations in the ATP binding domain of the kinase (160). Crystalographic studies which aim to elucidate the architecture of the mutated proteins are providing insight into the process of resistance (161). Studies showing that 17-allylaminogeldanamycin (17-AAG), an inhibitor of the molecular chaperone heat shock protein 90 (Hsp90) (in itself a modulator of Brc-Abl), induces degradation of wild-type and mutated (imatinib-resistant) hematopoietic cells, are very encouraging for possible combination studies (162). When proteins, including Bcr-Abl, Fak, ErbB2, mutant p53 and Raf-1, associate with heat shock proteins they become stabilized. In the case of Bcr-Abl, the disruption of these heterocomplexes by the Hsp90 inhibitor causes rapid degradation of the Hsp90-client protein by the proteosome.

Monoclonal antibodies and other approaches to tyrosine kinase inhibition

In general, the efficacy of antityrosine kinase antibodies stems from their ability to inhibit ligand binding and hence receptor dimerization. Recombinant antibody technology has enabled the design, selection and production

of humanized or human antibodies, human-mouse chimeric or bispecific antibodies for targeted cancer therapy (163). Evidence also suggests that by cross-linking or binding to membrane receptors, monoclonal antibodies are able to alter the receptor signaling activity, resulting in induction of proapoptotic signals (164). Trastuzumab (Herceptin®; Genentech), approved by the FDA in 1998 for the treatment of ErbB2 overexpressing breast tumors, and cetuximab (Erbitux™, IMC-C225; ImClone Systems), now in phase III trials, represent the two most developed, antibody-based tyrosine kinase inhibition approaches. Trastuzumab, which represents the first genomic-based therapeutic agent that is applied selectively on the basis of genetic characteristics of the tumor, can induce tumor responses when given alone and enhances the effectiveness of several chemotherapeutic agents and is now being tested in adjuvant settings (165-168). It is, in addition, one of the first successful applications of "theranostics", *i.e.*, the development of diagnostic tests directly linked to the application of a specific therapy. Simultaneous approval was granted by the FDA for both trastuzumab and DAKO's HercepTest for the diagnosis of ErbB2 overexpression (169). Interstudy variability issues have raised significant concerns, however, since initial retrospective analysis attempting to relate the level of ErbB2 expression to clinical response in patients treated with trastuzumab have been inconsistent and unconvincing (170, 171).

The monoclonal antibody cetuximab, an EGFR targeting molecule, displayed significant antitumor activity in various *in vivo* tumor models (pancreatic, breast, renal tumors) (172-174). Phase II clinical trials revealed that it is well tolerated as a single agent and when combined with chemotherapy or radiotherapy. It possesses a manageable toxicity profile (transfusion-like symptoms have been reported, probably a toxicity that is common to all antibodies) and it has been tested in combination with standard therapies in patients with various stages of colorectal cancer and pancreatic cancer (175-177). However, as of January 2003, the FDA refused to accept filing of the Biologics License Application (BLA) for cetuximab for the treatment of irinotecan-refractory colorectal cancer. Since then, the BOND phase II trial has showed that the combination of cetuximab and irinotecan reduced tumors in more patients and delayed tumor progression longer than cetuximab alone in patients with metastatic colorectal cancer that had progressed after treatment with standard irinotecan-based chemotherapy. Cetuximab still awaits approval for marketing in both the U.S. and Europe (http://www.cancer.gov/clinical trials).

The approval by the FDA of the immunotoxin ONTAK (DAB389IL2) for cutaneous T-cell lymphoma (CTCL) and encouraging results described with a number of diphtheria (DT) conjugates for different neoplasms suggest that a niche is developing for these biologic agents (178, 179). Immunotoxins are toxins fused or chemically conjugated to a specific ligand, such as the variable domains of heavy and light chains of monoclonal antibodies, or to growth factors. Rapid internalization of the conjugate follows upon binding to the specific cell surface receptor and once inside the cell cytoplasm, it directly inhibits protein synthesis and leads to cell death (179). Tumor cells carry specific antigens recognized by these complexes and are therefore targeted, in contrast to healthy cells. The DAF389EGF immunotoxin, now in phase II clinical trials, contains the enzymatically active and membrane translocation domains of the DT toxin and sequences of the human EGF and has been shown to inhibit growth in several experimental tumor systems (180).

In parallel to more conventional therapeutic approaches, various antisense strategies are being evaluated in preclinical studies and not only in malignant conditions (181-184). Antisense oligonucleotides targeting IGF-1R induced apoptosis of melanoma cells and reversal of epidermal hyperproliferation related to psoriasis (181, 182). This strategy is now being evaluated in the clinic for the treatment of brain tumors.

Novel potential tyrosine kinase targets

In addition to all of the compounds already in clinical development, numerous other tyrosine kinase inhibitors are in preclinical development. In most cases these are second-generation, follow-up compounds with optimized chemistry and pharmacology and more efficient evaluation in already validated tumor indications. At the same time, considerable effort is dedicated to the identification of novel or further ratification of existing tyrosine kinase targets. The ability to detect activating mutations and the presence of these in particular types of disease appears to be pivotal in the preclinical and clinical development of tyrosine kinase inhibitors. On the other hand, detection of gene amplifications, in the absence of activating mutations, also provides evidence for the role of specific tyrosine kinases in disease. This, however, is not direct proof since the detected amplicons may encode multiple proteins. For example, CDK4 and MDM2 in gliosarcoma, ErbB2 and c-Myc in breast cancer and prostate cell antigen and c-Myc in prostate cancer, colocalize in the same amplicon (185-187). Coamplification might hinder the significance of amplification of a particular locus, although this problem can be overcome with the use of high-resolution mapping of amplicons (185). Most targets of tyrosine kinase inhibitors currently in clinical trials have been shown to be mutated in at least one type of human cancer. Similarly, other tyrosine kinases have been found mutated in a variety of human tumors and are potential objects for inhibition. These include Met in renal carcinomas and Ret in thyroid carcinomas and multiple endocrine neoplasia syndromes. Kinase overexpression has been described for Scr in mammary and pancreatic cancers, IGR-1R in cervical carcinomas, as well as the angiogenesis-related receptors, Tie1 and Tie2, in hemangioblastomas and gastric adenocarcinomas and EphA2, EphB2, EphB4 in melanomas, gastric, esophageal, colon and mammary carcinomas.

Met, the physiological role of which is not completely understood, is commonly overexpressed in tumors and point mutations have been identified in hereditary and sporadic papillary renal carcinomas, gastric, hepatocellular and head and neck carcinomas. The fact that it has additionally been implicated in tumor metastases makes it a very attractive target candidate (188, 189). Ret tyrosine kinase is normally required for the development of kidneys and the enteric system and for neuronal differentiation and survival. Somatic Ret function mutations are found is some sporadic tumors while germline mutations are involved in 3 familial tumor syndromes: multiple endocrine neoplasia 2A and 2B (MEN2A, MEN2B) and familial medullary thyroid carcinoma (190). All identified oncogenic mutations cluster in extracellular, highly conserved regions of the Ret PTK domain that are normally involved in kinase repression in the inactive receptor (191). It is interesting that the frequently mutated Asp816 in Kit is a highly conserved residue just C-terminal to the conserved Asp-Phe-Gly motif in the activation loop of protein kinases. Mutations of the corresponding residue in Met and Ret result in papillary renal and thyroid carcinomas, respectively, and shift the equilibrium of the activation loop in unstimulated RTKs towards the active conformation (191, 192).

Mutated Scr resulting in a truncated form of the protein has been implicated in human colon cancer and mutated/overexpressed receptor in mammary, pancreatic cancer and neuroblastomas (193-195). The need to avoid targeting Lck, a Scr family kinase critically involved in T-cell physiology, has been an obstacle in the development of Scr selective inhibitors. ATP mimetics with approximately 10-fold selectivity for Scr over Lck have been developed. Efforts to develop phosphotyrosine mimetics in order to inhibit protein-protein interactions are also under way (197-200).

The IGF-1R family, together with the EGFR family, exemplifies how autocrine/paracrine loops activate the signaling machinery (41, 42, 201, 202). Elevated levels of IGF-IR are observed in a variety of human tumor types, whereas epidemiological studies implicate the IGF-1 axis as a predisposing factor in the pathogenesis of human melanoma, brain and cervical cancers (203-205). The IGF-1R is widely expressed across many cell types in fetal and postnatal tissues. Activation of the receptor following binding of the secreted growth factor ligands, IGF-1 and IGF-2, elicits a repertoire of cellular responses including proliferation and the protection of cells from apoptosis (206). Forced overexpression of the IGF-1R results in the malignant transformation of cultured cells and is essential for anchorage-independent growth (207, 208) Conversely, downregulation of IGF-1R levels can reverse the transformed phenotype of tumor cells and may render them sensitive to apoptosis *in vivo* (207). Thus, inhibitors of IGF-1R should render cells more sensitive to undergo apoptosis and reduce their metastatic potential. Proof of concept validation has been achieved using antisense methods, anti-IGF-1R antibodies and dominant negative mutants. However, no ATP site specific IGF-1R tyrosine kinase inhibitor has been described in the literature (209).

RTKs have emerged as critical mediators of angiogenesis and therefore such family members that are specific to the endothelium have drawn great attention. These primarily include the Tie and Eph receptors. The Tie family of receptor tyrosine kinases (Tie1 and Tie2) are specific and vital to angiogenesis and have been found overexpressed in various human tumors, including hemangioblastomas and hemangiopericytomas, gastric adenocarcinoma and NSCLC (18, 210). No ligands for Tie1 have been identified yet, whereas Tie2 is bound by the angiopoietins (Ang1, Ang2 and Ang4 in humans) (211-213). Binding of Ang1 and Ang4 causes activation of the receptors with subsequent stabilization and maturation of the vasculature and additional antipermeability effects. However, Tie2 mostly acts as a natural antagonist and its binding to the same receptor inhibits activation by Ang1 (212). This dual effect is mediated by separate ligands on the same receptor and is unprecedented in vertebrates and elucidation of the exact molecular details would be of immense importance in the receptor protein and angiogenesis research. In addition, it is possible that manipulation of the same receptor can yield positive results in both, proangiogenic and antiangiogenic conditions. Targeting of Tie2 using gene therapy approaches (*e.g.*, adenoviral vector carrying soluble receptor) has resulted in tumor inhibition (214). The intracellular domain of Tie2 has been cocrystallized and small number of compounds has been identified as inhibitors (215).

The Eph receptors mediate cellular repulsion, adhesion, cell attachment and migration in embryonic patterning, neuronal targeting, vascular development and adult neovascularization (216). They interact with surface bound ligands and initiate signaling in a bidirectional fashion (216). Deregulation of Eph receptors has been shown to affect cell survival or promote abnormal growth, to result in loss of cellular adhesion and to modulate cell-cell and cell-matrix attachment (217-219). EphA1 and EphA2 (and its ligand ephrin A1) have been found to be overexpressed in a variety of human tumors, including melanoma and prostate cancer, during neovascularization (217, 220, 221). Furthermore, overexpression of EphA2 in normal breast epithelial cells gives rise to malignant tumors in athymic mice (222). Inhibition of the EphA class of receptors using soluble receptors led to inhibition of tumor progression *in vivo* via inhibition of tumor angiogenesis (223).

Conclusions

There are just over 90 anticancer drugs currently approved by the FDA, the majority of which are indiscriminate poisons of the cell replication machinery and several of them are more than 40-50 years old (http://www.accessdata.fda.gov/scripts/cder/onctools/statistics.cfm). However, a relatively recent study by the World Health Organization concluded that curable

cancers and those cancers where the cost-benefit ratio clearly favors drug treatment can be managed with regimens based on only 17 drugs (and all of these are available at relatively low cost as generic preparations) (224; http://www.who.int/medicines/organization/par/edl/infedl11α.html). In addition, surgery still remains the main anticancer modality accounting for at least 80% of cancer "cures" while cancer chemotherapy is effective in only 10% of all cancers. At the same time, the death rate from cancer is expected to increase from 6 to 10 million by the year 2020 (World Health Report, 2000). Therefore, the need for new, efficacious cancer therapy is clearly evident. Kinase inhibitors represent an innovative approach that is moving the field of cancer chemotherapy forward. There are numerous issues emerging from the studies and relatively recent experience with tyrosine kinase inhibitors. One of these is the selection of the appropriate kinase target. Some kinases might have pivotal roles in the pathology of cancer by virtue of mutational activation, whereas other kinases might have an amplifying or permissive role in deregulated growth. However, mutated (or amplified) kinases have not yet been identified for most solid tumors and the majority of solid tumors exhibit multiple aberrant signaling pathways and may not express and/or be driven by mutated kinases (33). Advances in technologies such as DNA microarrays, bioinformatics and proteomics which aim to provide knowledge on the molecular signatures and construct molecular interaction maps of diseases can have a great positive impact (225-227). A recent large-scale, sequence-based study in a series of colorectal cancers identified 46 somatic, previously unknown, mutations in 15 tyrosine kinase genes. In addition, it was suggested that a minimum of 30% of colorectal cancers contain at least one mutation in the tyrosine kinome (228).

Exploiting other areas of kinase inhibition rather than the ATP binding site can provide novel targets. Developing inhibitors that affect: a) the maturation of the newly synthesized protein to the mature correctly folded kinase, b) the efficiency of translation of the kinase itself or of its activating regulators, c) the transcription of kinase genes or of their regulators, d) the stability of the kinase itself or competition with its substrate and e) phosphatase action, leading to accelerated removal of substrate phosphates or as a means of countering kinase signaling (229). Some of these possibilities are at the moment purely theoretical, but others are already possible and are being tested preclinically and even in the clinic, as mentioned earlier.

A significant contribution in the field of tyrosine kinase inhibition has been the extremely valuable knowledge obtained in relation to clinical research. Appreciation of how this should be adapted to a specific drug tested, and the fact that for this type of agent molecular effects might be more relevant than macroscopic observations, should be considered. Additional experience has been gained on issues regarding single or combination therapy which include the selection of patients such as those who would most benefit from this treatment, the establishment of preclinical pharmacokinetic/pharmacodynamic relationships for translation into the clinic, issues regarding side effects (mechanism- or nonmechanism-based) and the emergence of resistance. For all of these issues, different evaluation criteria have to be set in comparison to conventional, cytotoxic therapy. Selection of patients relates to selection of the kinase target and is extremely important in order to examine the actions of a compound preclinically and determine its benefits as a drug. An important aspect of the determination of PK/PD relationships is the fact that in contrast to many cytotoxic agents that interact with their targets covalently or in a poorly reversible fashion, kinase inhibitors must be present constantly and above a certain K_i to effectively compete with ATP. Doses should therefore be optimized according to intratumoral effects. This also implies the need to define and effectively use disease-specific biomarkers in order to aid diagnosis, monitoring and treatment. It appears that reported toxicities (rash and diarrhea) for tyrosine kinase inhibitors are also common to other kinase inhibitors and can be attributed to inhibition of signaling events downstream of the EGFR $\rightarrow$ Raf $\rightarrow$ MEK $\rightarrow$ ERK signaling axis. This can provide valuable information in further and new development of such agents. Resistance to tyrosine kinase inhibitors can emerge by a number of different mechanisms (new mutations, amplifications, redundancy/elimination of relevant signaling pathways, upregulation of drug efflux pumps) and has already been described for imatinib. Interestingly, resistance to imatinib in some cases is thought not to represent true development of resistance but rather selective outgrowth of preexisting subclones of CML cells (160, 230, 231). Whether this is the case for other kinase inhibitors or if nonspecific inhibitors are less likely to succumb to resistance, remains to be seen.

Finally, a major disappointment in the field has been the lack of beneficial effects of gefitinib in combination with standard cytotoxics in the INTACT trials, as it had been demonstrated in preclinical models. On one hand, this can be attributed to the drug itself or in the design of the clinical trials and the various issues associated with this. Fundamentally, though, it also questions the validity and true predictability of our preclinical models and our knowledge of their biology. Our knowledge of the cell's (normal or diseased) molecular circuit has progressed but is still limited. Considerably greater understanding of cell biology is required to optimally exploit kinase-targeted therapies in cancer or other diseases. Tyrosine kinases are and will be providing us with the tools to further understand cell biology and develop new concepts in disease therapy.

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