Smart drugs: Tyrosine kinase inhibitors in cancer therapy

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Cancer therapy directed at specific, frequently occurring molecular alterations in signaling pathways of cancer cells has been validated through the clinical development and regulatory approval of agents such as Herceptin for the treatment of advanced breast cancer and Gleevec for chronic myelogenous leukemia and gastrointestinal stromal tumors. While most novel, target-directed cancer drugs have pregenomic origins, one can anticipate a postgenomic wave of sophisticated "smart drugs" to fundamentally change the treatment of all cancers. With these prospects, interest in this new class of therapeutics extends from basic research scientists to practicing oncologists and their patients. An extension of the initial successes in molecular oncology will occur more quickly and successfully through an appreciation of lessons learned with the first group of agents in their progress through clinical development.

Introduction

Herceptin (trastuzumab) for the treatment of metastatic breast cancer and Gleevec (imatinib mesylate) for the therapy of patients with Philadelphia chromosome-positive (Ph^+) chronic myelogenous leukemia (CML) are the first examples of gene-based cancer drugs, and represent the most significant developments toward a new era of target-directed therapies. These agents not only prolong life and improve its quality, they also provide clinical validation of the emerging field of molecular oncology, specifically therapies targeting kinase enzymes that play a critical role in tumorigenesis. The lessons learned with Herceptin and Gleevec and with late stage development agents directed at the tyrosine kinase activity of the epidermal growth factor receptor (EGFR) and vascular endothelial growth factor receptor (VEGFR2) have caused a general shift in strategy and tactics in drug development. This article reviews those lessons and their application in the development of other agents, targeting these and other kinases that have more recently entered the development pipeline (Table 1).

HER2/*neu* as breast cancer target

The breakthrough discovery of Bishop and Varmus that cancer-inducing genes of animal retroviruses such as v-src or v-ras represent mutated host genes that were recombined into the viral genome raised the question of whether the oncogene concept was also relevant to human cancer (Varmus and Bishop, 1986). The first cloning and sequence analysis of a cDNA encoding a cell surface protein, the human EGF receptor, provided a partial answer to this question by revealing a close relationship with the v-erbB oncogene (Downward et al., 1984; Ullrich et al., 1984). This first connection between a human gene product that regulates normal cell proliferation and a viral oncogene strongly suggested that human cancer development may also involve abnormalities in the expression and structure of endogenous genes that have regulatory roles in cell proliferation. A search for such genetic aberrations in tumor tissues using cDNA probes of EGFR and an accidentally cloned EGFR-related gene, termed HER2 (human EGFR-related gene), resulted in the discovery that the gene encoding the HER2/*neu* receptor tyrosine kinase is amplified up to 100-fold in the tumor cells of about 30% of patients with invasive breast cancer. A significant clinical correlation was shown between HER2/*neu* gene amplification and overexpression and parameters of malignancy, including reduced survival and reduced time to relapse, relative to patients with normal receptor levels (Slamon et al., 1987; Chazin et al., 1992).

These findings led to the development of Herceptin (anti-HER2 Mab; Trastuzumab; Genentech), the first genomic research-based, targeted anti-kinase therapeutic approved for cancer therapy. It was designed by humanizing the mouse monoclonal antibody 4D5 (Fendly et al., 1990) that binds HER2/*neu* on the surface of tumor cells, thereby inducing receptor internalization and inhibition of cell cycle progression through mechanisms that include upregulation of $p27^{kip1}$, an intracellular inhibitor of cyclin-dependent kinase 2 (CDK2) (Carter et al., 1992; Shepard et al., 1991; Rieske et al., 1999). In clinical trials with patients with metastatic breast cancer, objective responses were seen in previously treated patients (15%) with Herceptin used alone (Cobleigh et al., 1999), and particularly encouraging efficacy was seen with Herceptin in combination with standard chemotherapy (Slamon et al., 2001). Demonstration of the antitumor activity of Herceptin against breast cancer provided the proof of principle for therapy targeted to receptor tyrosine kinases (RTKs), a class of cell surface receptors with an intrinsic intracellular tyrosine kinase activity that includes Her2/*neu*, EGFR, IGF-1R, PDGFR, CSF-1R, c-kit, Flt 4, FGFR, Met, and Tie-2, all of which have been identified as potential therapeutic targets in cancer therapy (Shawver, 1999).

HER2/*neu*-overexpressing breast cancer cells are readily identified by immunohistochemistry and/or fluorescence in situ hybridization (FISH), allowing selection of patients that are likely to respond to Herceptin therapy. However, since breast cancer and most other types of cancer are believed to be caused by multiple genetic events, a key to success in treating these cancers will be found in a rational combination of agents, each of which contributes uniquely to the elimination of cancer cells. Breast tumors that overexpress HER2/*neu* are less responsive to chemotherapy. Experimental studies have shown that HER2/*neu* overexpression confers resistance to taxanes and that Herceptin enhances the response of HER2/*neu*-overex-

Table 1. Tyrosine kinase targeted therapies

Common name	Trade name	Target	Indication[a]	Company
Gleevec	Gleevec	Brc-Abl,c-kit, PDGFR	CML,GIST	Novartis
Herceptin	Herceptin	Her2/*neu*	Breast cancer	Genentech
Cetuximab	Erbitux	EGFR	Colorectal cancer	Imclone
ZD1839	Iressa	EGFR	NSCLC	AstraZeneca
OSI-774	Tarceva	EGFR	Pancreatic cancer	OSI/Genentech/Roche
PKI 166	NA/ND	EGFR	+	Novartis
GW2016	NA/ND	EGFR	+	GlaxoSmithKline
CI-1033	NA/ND	EGFR	+	Pfizer
EKB-569	NA/ND	EGFR	+	Wyeth
Semaxanib	NA/ND	VEGFR2	Colorectal cancer	SUGEN/Pharmacia
ZD6474	NA/ND	VEGFR2	+	AsraZeneca
PTK-787	NA/ND	VEGFR1, R2	+	Novartis/Schering AG
INC-1C11	NA/ND	VEGFR2	+	ImClone

+, agents in early clinical development; CML, chronic myelogenous leukemia; EGFR, epidermal growth factor receptor; GIST, gastrointestinal stromal tumors; NA/ND, not available/not determined; NSCLC, non-small cell lung cancer; VEGFR2, vascular endothelial growth factor 2.
[a]Approved indication or primary indication sought. Other potential indications are referred to in the text.

pressing breast cancer cells to taxanes as well as to anthracyclines and platinum compounds (Yu, 2001). In a phase III comparison trial with Herceptin added to first-line therapy with anthracycline-cyclophosphamide or paclitaxel in patients with HER2/*neu*-overexpressing metastatic breast cancer, the benefit obtained with Herceptin was significantly greater than that achieved with the standard therapy alone. Median survival was significantly increased (25.1 versus 20.3 months, P = .046), with a 20% overall improvement of survival (Slamon et al., 2001). On the basis of these findings, Herceptin received regulatory approval in the United States for use with paclitaxel in the first-line treatment of patients with HER2/*neu*-overexpressing metastatic breast cancer. Herceptin is also approved as a single agent in the treatment of patients with HER2/*neu*-overexpressing metastatic breast cancer who had received at least one prior cycle of chemotherapy for metastatic disease. Ongoing studies with Herceptin are extending its use in breast cancer to the adjuvant setting and with other combination chemotherapy regimens. Moreover, second-generation Herceptin therapies, such as the cytotoxic conjugate Herceptin-DM1 and the monoclonal antibody 2C4 (Fendly et al., 1990) have shown promise in preclinical testing.

The Herceptin clinical trials were the first studies to include patients who shared a genetic abnormality in their tumor cells (HER2 gene amplification), and who were therefore likely to respond to target-specific therapy. These trials also showed that a targeted therapy may be effective as a single agent. However, the efficacy of Herceptin and that of standard chemotherapy may be enhanced by combination. Identification of the optimally effective combination regimens remains an important objective in the era of molecular therapeutics. It is likely that future cancer therapies will involve a combination of target-specific agents that are aimed at different hallmark characteristics of tumor cells, such as hyperproliferation, angiogenesis, invasiveness, and antiapoptosis, rather than traditional cytotoxic compounds.

Targeting the Bcr-Abl kinase: Gleevec (STI571; Imatinib mesylate; Novartis)

As described in a recent review by B.J. Druker (Druker, 2002), the p210 Bcr-Abl is a constitutively active cytoplasmic tyrosine kinase present in virtually all patients with CML and 15% to 30% of adult patients with acute lymphoblastic leukemia (ALL). It is a model target for cancer therapy, because the Bcr-Abl tyrosine kinase by itself is sufficient for the development of leukemia, and all transforming activities of Bcr-Abl depend on this enzymatic activity (Daley et al., 1990; Heisterkamp et al., 1990; Kelliher et al., 1990). Bcr-Abl is created through a reciprocal translocation between chromosomes 9 and 22, which results in a fusion between the genes encoding the *abl* tyrosine kinase and *bcr* (Rowley, 1973; de Klein et al., 1982). This translocation generates the distinctive Philadelphia chromosome, an easily identified marker for CML. Gleevec is a low molecular weight inhibitor of the tyrosine kinase activity of Bcr-Abl, as well as that of Abl, PDGFR, and c-kit, the receptor for stem cell factor. Gleevec blocks adenosine triphosphate (ATP) binding to the kinase, preventing phosphorylation reactions required for transduction of signals that stimulate cell proliferation and inhibit normal mechanisms of cell death, indefinitely extending the life of the malignant cells. Clinical trials with Gleevec confirmed the validity of Bcr-Abl as a target in CML. In phase II studies with 532 patients with chronic phase CML, Ph^+ cells were completely cleared from the circulation of nearly all (95%) patients, all of whom had previously failed standard interferon therapy. These hematologic responses usually occurred within 4 weeks and were durable, with only 9% of patients relapsed at a median followup interval of 18 months (Druker, 2002). The regulatory application for use of Gleevec in CML in patients in blast crisis, accelerated phase, or in chronic phase after interferon failure was approved within 3 months of submission. The speed of passage of this agent through clinical trials and regulatory review has become a case study in rapid drug development.

Gleevec was most effective against CML in patients in the early chronic phase of the disease. Among patients in myeloid blast crisis, there were fewer responses (52%), and most (78%) responding patients relapsed within 1 year. Relapse coincided with point mutations in the *bcr-abl* kinase domain in about half the relapsed patients, and in a third of patients, relapse could be ascribed to *bcr-abl* gene amplification and kinase overexpression (Gorre et al., 2001; Hochhaus et al., 2001; Shah et al., 2001). De novo resistance correlated with more advanced disease and was possibly related to accumulation of resistance-conferring mutations during disease progression (Druker, 2002). In the treatment of CML with Gleevec, clinical efficacy is highly correlated with early initiation of therapy, a lesson that will

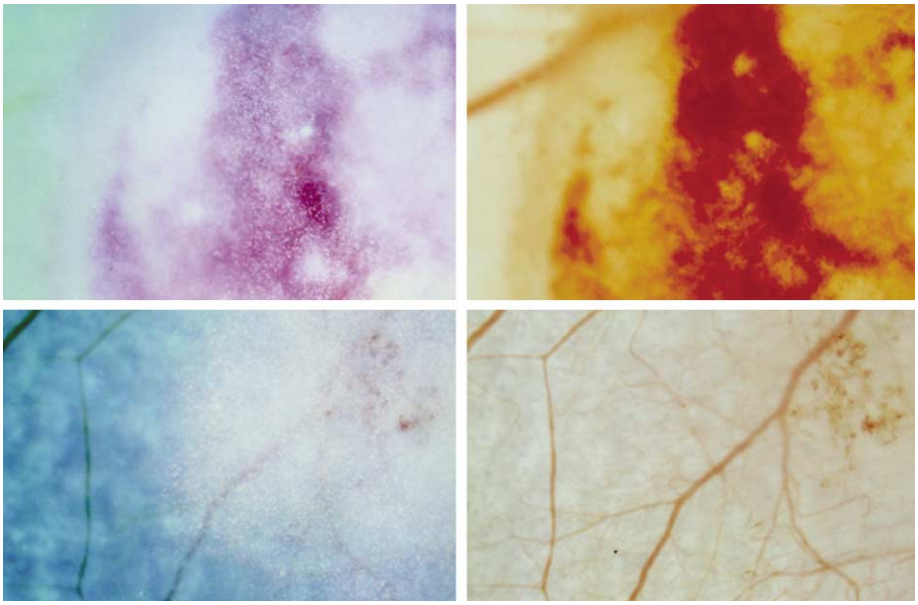

Figure 1. Semaxanib efficacy in a tumor model

C6 gliomas on day 4 after glioma cell implantation and daily treatment with saline/DMSO (50 μl/day, IP, top panels) or SU5416 (25 mg/kg/day, IP, bottom panels) visualized by intravital microscopy applying transillumination (right panels) and combined trans- and epiillumination techniques (left panels). Tumor cells are stained with Fast Blue (Sigma), allowing exact identification of tumor mass. Reprinted with permission from Vajkoczy et al. (1999).

likely apply to other types of cancer as well (Allan et al., 1995; Druker, 2002).

The success of Gleevec in CML was extended quickly to gastrointestinal stromal tumors (GIST) in which an activating mutation in the gene encoding the receptor tyrosine kinase c-kit has been identified as a pathogenic event. Remarkable clinical activity was seen in this chemotherapy-refractory tumor, leading to approval in 2002 for use in patients with advanced GIST (Blanke et al., 2001; Van Oosterom et al., 2001). Activating c-kit mutations also occur in acute myeloid leukemias (Kanakura et al., 1993), small cell lung cancer (Hibi et al., 1991), neuroblastoma (Cohen et al., 1994), germ cell tumors (Strohmeyer et al., 1991), and breast cancers (Hines et al., 1995), although it is not known whether c-kit activation is a critical event in these tumors. Clinical trials will determine the importance of activated c-kit in these cancers and the significance of this activation with respect to efficacy of treatment with Gleevec (Druker, 2002).

Targeting the epidermal growth factor receptor

The receptor for epidermal growth factor is one of 4 members of an RTK subfamily that also includes HER2, HER3, and HER4. The first member of this family to be sequenced and described (Ullrich et al., 1984), the EGFR (c-erbB1), is broadly expressed in a variety of cells of ectodermal or mesodermal origin (Borg et al., 2000). Overexpression of EGFR commonly occurs in many types of cancer, such as head and neck, non-small cell lung (NSCLC), laryngeal, esophageal, gastric, pancreatic, colon, renal cell, bladder, breast, ovarian, cervical, prostate and papillary thyroid cancers, melanoma, and gliomas, and correlates with a poor clinical outcome (Nicholson et al., 2001). Signaling through the EGFR activates pathways that stimulate many of the properties associated with cancer cells: proliferation, migration, stromal invasion, tumor neovascularization, and resistance to cell death-inducing signals (Schlessinger, 2000). The principal EGFR ligands are EGF and transforming growth factor-α (TGF-α), heparin binding EGF (HB-EGF), amphiregulin, betacellulin, and epiregulin. Tremendous signaling diversity occurs in the EGFR family through heterodimer formation among family members and activation by various splice forms of Heregulin ligands (Yarden and Sliwkowski, 2001).

The first efforts to target EGFR in cancer therapy by Mendelsohn and colleagues used a monoclonal antibody to extracellular epitopes of the receptor, and clinical evaluation of this antibody is still underway (C225; Cetuximab) (Kawamoto et al., 1983). In recent years, several pharmaceutical companies have developed small-molecule inhibitors of EGFR tyrosine kinase activity. Several agents are currently in clinical development, differing in their specificity for EGFR and other members of the HER2 family, their potency, and the reversibility of their interaction with the ATP binding site in the kinase domain of the receptor (Table 1). As these agents progress through clinical development, more will be learned regarding the value of these differences in determining the overall efficacy and usefulness. Among the EGFR inhibitors in clinical development, Iressa (ZD1839, AstraZeneca) has progressed furthest, and its development is influencing that of other targeted therapeutics.

Iressa, a small molecule ATP binding site inhibitor like Gleevec, exhibits growth inhibitory effects against a wide variety of human tumor xenografts, although tumor regrowth typically follows termination of treatment (Ciardiello et al., 2000). Growth inhibition is associated with cell cycle arrest mediated through upregulation of $p27^{Kip1}$(Budillon et al., 2000), the same mechanism proposed for Herceptin-mediated inhibition of cell proliferation through HER2/*neu* (Browder et al., 2000). As with Herceptin, Iressa enhanced the antitumor effects of taxanes and platinum compounds in preclinical animal studies (Ciardiello et al., 2000), but in contrast to Herceptin, the efficacy of Iressa is apparently not directly related to the level of EGFR expression (Albanell et al., 2001).

Iressa has progressed rapidly in a development program that early recognized the significance of its activity in managing patients with NSCLC, both as a single agent in patients whose disease was refractory to other therapies and in combination with standard regimens in the first-line setting. In single-agent trials, partial responses, minor responses, and disease stabilization were reported in about 25% of patients. Notable responses were seen among patients with advanced NSCLC who had received extensive prior chemotherapy (Ferry et al., 2000; Baselga et al., 2000). Accelerated regulatory approval is being sought for Iressa as a single agent in the treatment of patients with metastatic NSCLC who have failed standard therapy.

Identifying a clinical setting in which a new agent may provide substantial improvement over current therapy presents an

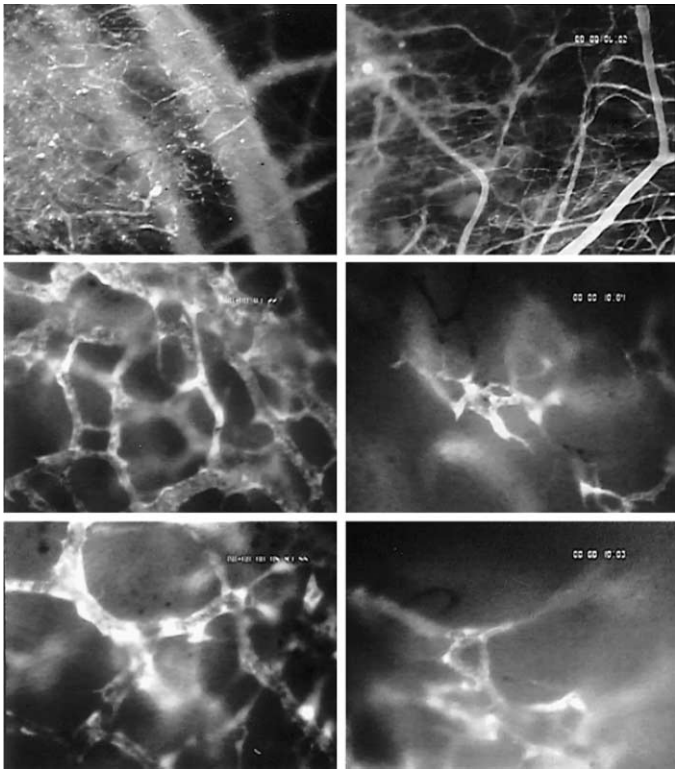

Figure 2. Semaxanib inhibits tumor angiogenesis in tumor xenografts

C6 glioma microvasculature in animals treated with DMSO (50 μl/day, IP; left row) or SU5416 (25 mg/kg/day, IP; right panels) on day 6 (top panels) and 18 (middle and bottom panels) after glioma cell implantation. Intravital multifluorescence videomicroscopy; contrast enhancement with 2% FITC-dextran$_{150}$ IV. Reprinted with permission from Vajkoczy et al. (1999).

opportunity for accelerated regulatory approval. The rapid progress of Iressa and Gleevec have established a new pattern for targeted therapeutics in clinical development, which focuses on an identified unmet clinical need and progresses from an initial phase I/II clinical trial to registration in 4 to 5 years, compared with the 7 or more years required with a conventional program of phase I/II/III development.

Breast cancer patients whose tumors express EGFR and overexpress HER2/*neu* have more aggressive disease than those with tumors that do not overexpress HER2/*neu*. Therapeutic strategies directed at these particularly aggressive breast cancers include combinations of agents specific for EGFR and HER2/*neu*, as well as single agents that target the kinase activity of both receptors. The first combination of targeted therapeutics to enter clinical trials is Iressa and Herceptin in patients with metastatic breast cancer (Moulder et al., 2001). Agents such as CI-1033 and EKB-569 that inhibit both EGFR and HER2 may also be particularly effective against these cancers, although results of such trials have not been reported yet.

Targeting the VEGFR2 (KDR): Antiangiogenic therapy

Angiogenesis has been the subject of recent review articles (Fox et al., 2001; Liekens et al., 2001). The Folkman laboratory and, in recent years, a host of scientists and clinical investigators have established the essential nature of tumor angiogenesis in expansive cancer growth (Folkman, 1999). Antiangiogenic strategies direct therapy at the developing tumor neovasculature, rather than the tumor cell, and have attractive advantages, including reduced likelihood of genetic drug resistance and broad application among different types of cancer. More than 40 agents that principally target neovascularization are currently under clinical investigation in over 60 clinical studies. About a third of the molecular therapeutics in clinical development are directed against vascular endothelial growth factor (VEGF) or its principal receptor, the RTK VEGFR2 (KDR). Other angiogenesis-relevant targets include the RTKs VEGFR3, FGF receptors, and Tie2 and the integrin $\alpha_v\beta 3$. VEGF, however, stands out as the growth factor most consistently associated with angiogenesis and with clinical outcome in a variety of malignancies (Pinedo and Slamon, 2000). Inhibiting the function of VEGFR2 in endothelial cells is sufficient to prevent tumor growth in experimental models (Millauer et al., 1994), and since VEGFR2 expression is limited to endothelial cells, the toxicity potential of any agent specifically directed toward inhibiting VEGFR2 activity is expected to be small (Ellis et al., 2001).

Among the VEGFR2 inhibitors, Semaxanib (SU5416, SUGEN/Pharmacia) has progressed furthest in clinical development. The lessons learned in development of this compound may be applicable to other antiangiogenic agents. Semaxanib competitively blocks ATP binding to the tyrosine kinase domain of VEGFR2, and thereby tumor angiogenesis, inhibiting the growth of xenografts established from a variety of human cancers (Fong et al., 1999). Experimentally, the antiangiogenic activity of semaxanib was demonstrated in a rat glioma model in which tumor growth and vascularization could be monitored by videomicroscopy (Figures 1 and 2). Semaxanib treatment of rats with a growing tumor reduced the density and permeability of vessels in the glioma compared with the untreated control animals (Vajkoczy et al., 1999).

Visual demonstration of antiangiogenic activity was important in establishing the in vivo angiogenesis inhibitory effects of semaxanib as the mechanism of growth inhibition in the animal model. While blocking neovascularization halts progressive tumor growth, it does not usually cause tumor cell death or induce the massive regressions required for an objective response in a clinical setting, at least not with only a few weeks of treatment. Establishing the optimum dose and regimen for reducing the blood supply to the tumor is a clinical problem not unique to antiangiogenesis, but one which appears to be especially critical in the development of this therapy. Efforts are being made to address this problem through the use of imaging technologies such as dynamic contrast-enhanced magnetic resonance imaging (MRI), computerized tomography (CT), positron emission tomography (PET) scanning, and ultrasound. As yet, these methods are not validated for reasons related to the variety of potential methods and absence of standardized protocols (Padhani and Neeman, 2001). Nevertheless, the need to apply these methods to measure blood flow through tumors and assess the biologic activity of cells within the tumor is well recognized. Most current trials include an imaging component to help demonstrate the biologic effect and provide the opportunity to improve the use of the imaging methodology.

In a pilot study with fluorouracil and leucovorin in previously untreated patients with advanced colorectal cancer, no dose-limiting toxicity ascribed to semaxanib was seen, objective tumor responses were obtained, and median survival in excess of 23 months suggested improved efficacy compared with standard fluorouracil-leucovorin therapy (Rosen et al., 2000; Miller et al., 2001). A randomized comparison trial of the same regi-

men, however, showed no statistically significant clinical benefit, although some patients showed striking responses. These studies with semaxanib have provided useful information on targeting tumor angiogenesis through the VEGF receptor. They have also helped define the optimal use of angiogenesis inhibitors as single agents in patients with minimal residual disease or in combination with other cancer therapies. For example, additive and supradditive effects have been reported in preclinical studies of antiangiogenic therapy combined with radiation (Gorski et al., 1999; Geng et al., 2001). This is a counterintuitive finding, in that a treatment reducing tumor blood flow and consequently the oxygen tension within a tumor would be expected to reduce, not enhance, the cytotoxic effects of radiation therapy. A proposed mechanism, however, involves inhibition of a radiation-induced survival response with increased production of VEGF by tumor cells. VEGF blocks the cytotoxic effects of radiation on the microvascular endothelial cells of the tumor through a mechanism involving upregulation of antiapoptotic mechanisms (Bcl-2) in the endothelium. The efficacy of radiation therapy with a VEGFR2 inhibitor added to the protocol may be equal to that associated with higher radiation doses (Kozin et al., 2001). This suggests the possibility of delivering an effective therapeutic dose to patients unable to tolerate usually effective, higher doses of radiation alone.

New targets and perspectives

Generated by the success of Gleevec and Herceptin, the impetus for development of new targeted therapeutics that hold some promise as targets in cancer therapy extends to RTKs beyond HER2/*neu*, EGFR, and VEGFR2. The PDGF receptor is an RTK primarily found on fibroblasts and smooth muscle cells and also expressed in the kidney, testes, and brain. In glioblastoma, a large proportion of tumor cells express both PDGF and PDGFR, creating an autocrine stimulatory loop believed to be important early in tumor development and progression. PDGF/PDFGR autocrine loops have also been reported in meningioma, melanoma, neuroendocrine cancers, and cancers of the ovary, pancreas, stomach, lung, and prostate (George, 2001; Ostman and Heldin, 2001). Among the PDGFR inhibitors, CDP860 (Celltech) is being tested in Phase I trials, while SU101 trials were terminated in phase III due to extreme pharmacokinetic variation in glioblastoma patients and in spite of impressive responses in some cases.

Met, the receptor for hepatocyte growth factor (HGF), is an RTK normally expressed by adult epithelial cells during cell migration and wound healing. Its relationship to cancer progression and metastasis has been recently reviewed (Jiang et al., 1999; van der Vort et al., 2000). Met is expressed by cancer cells of various origin in which both HGF and Met may be overexpressed. Activating mutations and gene amplification in the Met gene have been observed in some cancers. Experimentally, expression of HGF and Met or mutant Met conferred malignant properties on normal cells (motility, invasion, tumorigenicity). Clinically, HGF/Met overexpression has been shown to correlate with poor prognosis in several types of cancer (Longati et al., 2001).

In addition to the RTKs, several cytoplasmic kinases have properties that suggest key roles in the regulation of the cell cycle, gene transcription, motility, cell death, and metabolism. Several kinases at critical junctures within these regulatory pathways have been identified as potential targets of cancer therapy. Among these critical kinases are protein kinase C in the PI-3 kinase/AKT survival pathway; Src family kinases, Raf kinase, and MEK in the cell proliferation pathways; and the cyclin-dependent kinases regulating progression through the cell cycle. Inhibitors of several of these kinases are in preclinical or early-phase clinical development.

With the impressive results obtained with Herceptin and Gleevec, the stage is set for more demonstrations of the power of gene-based therapy development. After the completion of the human genome sequence and the advent of powerful methodologies such as gene and tissue array analysis, in combination with bioinformatics, an excellent basis has been generated for the identification of genetic abnormalities in cancer cells and the characterization of their pathophysiologic significance. One can anticipate that the results of these studies will generate a new wave of target-specific drugs which, due to the extensive complexity of pathogenic alterations in the cancer cell signaling network, will eventually be applied in specific combinations. These combinations will be based on the individual patient's genetic fingerprint, including the comprehensive gene expression and mutation analysis of the tumor as well as a single-nucleotide polymorphism profile of the patient's chromosomal DNA.

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