Targeted Cancer Therapy: Promise and Reality

Shoshana Klein and Alexander Levitzki

Department of Biological Chemistry, The Alexander Silberman Institute of Life Sciences, The Hebrew University of Jerusalem, Jerusalem, Israel

Signal transduction therapy for cancer targets specific molecular elements that are essential for survival of the tumor. Gleevec has a profound effect on early phase chronic myeloid leukemia because it inhibits the major driving factor of the tumor, BCR-ABL. Almost all other cancers depend on several factors, and blocking a single signal transduction factor is largely ineffective. Effective signal transduction therapy will entail finding the appropriate combination of signal transduction inhibitors for each cancer. We discuss the use of preclinical animal models to predict successful signal transduction therapy in the clinic, and conclude that their utility is limited.

I. WHAT IS SIGNAL TRANSDUCTION THERAPY?

Just 5 years have passed since Gleevec (imatinib/Gleevec/STI-571) revolutionized the treatment of chronic myeloid leukemia (CML). Other targeted drugs are also beginning to have an impact on cancer treatment

Advances in CANCER RESEARCH
0065-230X/07 $35.00

DOI: 10.1016/S0065-230X(06)97013-4

and prognosis. The new "targeted" cancer drugs have generated a lot of excitement and heightened expectations of miracle cures. In this chapter, we explore the development of signal transduction therapy for cancer. We discuss some of its pitfalls, and how we believe some of these pitfalls can be overcome.

Underlying many diseases are signaling pathways gone awry. The most visible examples come from cancer biology, where numerous chromosomal changes occur. These chromosomal anomalies include polyploidy, aneuploidy, and gross chromosomal rearrangements such as translocations. Less obvious changes include mutations in protooncogenes that convert them to oncogenes and loss-of-function mutations in tumor suppressor genes. Cumulatively, these genetic changes lead to cell immortalization and eventually to fully blossomed transformation into a metastatic cancer cell.

Currently, we are shifting from a "black box" approach to specific, targeted therapy for the treatment of cancer. Over the past two decades, it has become apparent that the many genetic and epigenetic changes in the makeup of the cell on transformation, although complex, can be defined biochemically. The identification of the specific signaling proteins involved in cancer and the biochemical definition of the signal transduction pathways gone awry form the basis for signal transduction therapy.

A premise behind signal transduction therapy is that cancer cells are particularly sensitive to inhibition of their overexpressed or hyperactivated signaling proteins (Fig. 1). This is because the complex, overlapping signal transduction networks of the healthy cell provide robustness: when one pathway is inhibited, the effect on the cell is minimized by the presence of alternative, redundant pathways. Redundancy is reduced in cancer cells, owing to the large number of mutations that these cells sustain. Thus, cancer cell survival and proliferation depend on a few hyperactive signaling pathways, an Achilles' heel that the therapist can attack. Efforts are being made to generate agents that target key signaling proteins with key roles in carcinogenesis.

II. TYPES OF SIGNALING INHIBITORS

In principle, molecular targets that are implicated in a range of tumor types should be the most attractive for drug development. Two main classes of signaling inhibitors are in current use: small molecules and antibodies. Recognizing the families of proteins involved in the initiation, progression, and spread of cancer enables us to think rationally about which pathways to manipulate in order to suppress the disease.

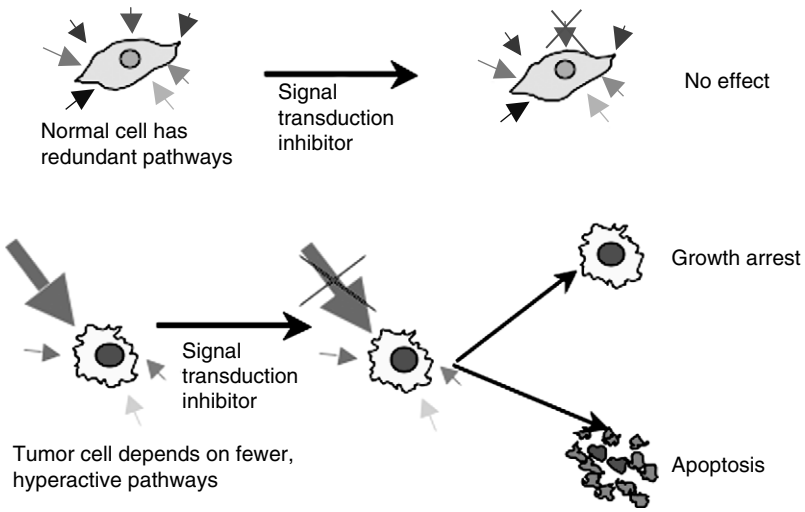

Fig. 1 The principle behind "signal transduction therapy": loss of redundancy, on the one hand, and hyperactivation of one or a few elements, on the other, renders the cancer cell exquisitely sensitive to targeted inhibition of the hyperactivated pathways.

A. Protein Kinases

The most popular candidates for targeted therapy are protein kinases (PKs). PKs play central roles in communication between and within cells. PKs control the balance between cell cycle progression, cell cycle arrest, and cell death and have been implicated in cancer, as well as in nonmalignant proliferative diseases, such as psoriasis and restenosis.

The human genome includes about 518 PKs. Ninety-one of these are protein tyrosine kinases (PTKs), comprising 59 receptor PTKs (RPTKs) and 32 nonreceptor PTKs. All of the PTKs and many of the serine/threonine kinases play key roles in communication between and within cells. RPTKs are transmembrane glycoproteins that transmit signals from outside the cell.

B. Targeting Cellular Proliferation

Many RPTKs are activated by growth factors and affect cellular proliferation. Examples of RPTKs with a known role in cancer include the epidermal growth factor receptor (EGFR) and the platelet-derived growth families factor receptor (PDGFR). Considerable experience has been assembled with EGFR inhibitors, which will be discussed below. Nonreceptor PTKs may be

activated by RPTKs, G-protein-coupled receptors, or immune receptors. Examples include the ABL and JAK families. Serine/threonine PKs such as RAF, ERK, and the cyclin-dependent kinases (CDKs) play key roles in proliferation and have been implicated in cancer. Many of the kinase inhibitors under development aim to inhibit cellular proliferation, thereby arresting tumor growth.

C. Targeting Cell Survival

The ability to escape apoptosis is a hallmark of most cancer cells and often correlates with tumor aggressiveness and resistance to traditional chemotherapeutic treatments. The serine/threonine kinase, PKB/Akt, promotes cell survival, and inhibitors of this protein are in preclinical development (Luo *et al.*, 2003). Nonkinase anti-apoptotic proteins that are candidates for signal transduction therapy include Bcl-2 and the inhibitor of apoptosis (IAP) family (Schimmer *et al.*, 2004).

D. Targeting Angiogenesis

Another strategy to arrest tumor growth involves curtailing blood supply to the tumor by inhibiting angiogenic factors such as vascular endothelial growth factor (VEGF) or its receptors. An antibody against VEGF, Avastin (bevacizumab), extends survival of patients with advanced colorectal carcinoma by several months on average, and has been approved by the FDA for the treatment of such patients, in combination with 5-fluorouracil (5-FU)-based chemotherapy (Hurwitz *et al.*, 2004).

E. Targeting Nuclear Factors

Many transcription factors are involved in promoting oncogenesis or regulating apoptosis. The estrogen receptor (ER) was the first example of successful targeting of a transcription factor. Indeed, the ER antagonist, tamoxifen, can be considered to be the first successful signal transduction inhibitor. Tamoxifen reduces the recurrence of estrogen-responsive breast cancer (Fisher *et al.*, 1998) and is now offered as a preventative measure to women at increased risk of contracting breast cancer.

The so-called "guardian of the genome," p53 (Lane, 1992), is mutated in over 50% of solid tumors, and p53 is inactivated by other means in many other tumors (Vogelstein and Kinzler, 2004). Reactivation of p53 appears to

reinstate apoptosis and render some tumors more responsive to radiation and chemotherapy (Cao *et al*., 2006; Coll-Mulet *et al*., 2006; Selivanova *et al*., 1998).

Another transcription factor that is being tested in preclinical studies is hypoxia-inducible factor 1α (HIF1α) (Kong *et al*., 2005; Kung *et al*., 2004; Powis and Kirkpatrick, 2004). Inhibiting angiogenesis is not effective against large, solid tumors, which are hypoxic and dependent on glycolysis, a phenomenon described by Otto Warburg in the 1930s (Warburg, 1956). HIF1α is essential for the switch to anaerobic metabolism and is also responsible for inducing VEGF (VEGFR). Thus, inhibiting HIF1α should starve the tumor both by preventing induction of glycolytic enzymes and by preventing angiogenesis and oxygen supply.

III. SIGNALING NETWORKS

Initially, we thought that signal transduction pathways were linear. The first signaling pathways to be elucidated, such as the hormone-dependent activation of adenylyl cyclase, indeed appeared to be linear. But we now know that each PK signals to a number of elements, which in turn signal to a number of downstream elements, such that a whole signaling network emanates from one initiator PTK. At any stage in this network, PTKs may be mutated into oncogenic forms. Extensive cross talk between signaling pathways serves to complicate the picture yet further. Some central signaling elements have multiple—and sometimes opposing—outputs, depending on cell or tumor type, environmental signals, and cellular status (Vermeulen *et al*., 2003). Ras is an example of a key signaling protein that can cause cellular proliferation, growth arrest, or cell death, depending on circumstances. The effects of inhibiting such an oncoprotein will need to be evaluated on a case-by-case basis.

There are only a few cases in which a disease can be linked to one major signaling event. For example, CML at its chronic phase is driven by BCR-ABL and can, therefore, be successfully treated by a BCR-ABL inhibitor (see below). An activating mutation in the *JAK2* gene (*JAK2*-V617F) has been found in many patients with another myeloproliferative disease, polycythemia vera, as well as in a large minority of patients with essential thrombocythemia (Schafer, 2006). Clonal chromosomal translocations or mutations that lead to activation of a specific tyrosine kinase are common in hematological cancers (De Keersmaecker and Cools, 2006), suggesting that these cancers in general might be susceptible to PTK inhibitors. Similarly, the nonmalignant development of restenosis following balloon angioplasty

is driven by PDGF and PGDFR, and therefore the process can be effectively halted by a stent eluting a PDGFR kinase inhibitor (Banai *et al*., 2004). Most solid tumors, on the other hand, are dependent on a conglomerate of oncogenic mutations, so a single inhibitor may not have a profound effect, even if its particular target is hyperactivated in a given tumor. Furthermore, the intrinsic genomic instability of cancer cells leads to continual evolution and to tumor heterogeneity so that a given inhibitor may target only a subpopulation of the tumor cells. Thus, our hope that inhibiting one or two major survival factors would lead to complete cancer cures has proven to be simple-minded.

IV. TARGET AND DRUG EVALUATION USING PRECLINICAL TUMOR MODELS

The shift from cytotoxic chemotherapy to targeted signal transduction therapy necessitates a shift in preclinical drug assessment strategies. In the quest for rationally developed drugs, target validation is a critical step toward verifying a purported mechanism of action. Thus, a drug should be tested on appropriate preclinical models in which the desired target is known to be expressed and to be important for cell/tumor survival.

Several studies have compared the efficacy of chemotherapeutic drugs on various preclinical cancer models with the results from clinical trials (Fiebig *et al*., 2004; Johnson *et al*., 2001; Voskoglou-Nomikos *et al*., 2003). The take-home message from these studies is that preliminary *in vitro* cytotoxicity tests using cell lines derived from cancer cells, together with secondary screens using mouse xenografts of human tumors (primary cells or cell lines), have some degree of use for screening candidate molecules for traditional chemotherapy. The more cell lines and xenografts display sensitivity to a drug, the more likely is the drug to show some level of activity in clinical trials.

A. *In Vitro* Screens

When designing targeted therapy, we have a specific molecular target in mind. Such projects often begin with an *in vitro* screen for inhibitors of that target. Large scale screens have been undertaken by many groups for specific, small molecular weight kinase inhibitors, using cell-free assays. Cell-free assays have the advantage that the molecular target is defined, but drugs that act on other components of the same signaling pathway will be

missed. Another advantage of cell-free assays is that the chemist can define the active portion of the lead compound and leave its pharmacological formulation to a later stage.

Alternatively, the initial screen will employ a cancer cell line or a genetically engineered cell line in which the target is known to be strongly activated because of either mutation or overexpression (or both). Lead compounds obtained either through cell-free screening or in cultured cells are then tested on additional cell lines, and inhibition of the target molecule must be confirmed by biochemical studies.

Good *in vitro* models of cancer would simplify screening for cancer drugs, as well as adding to our basic understanding of how cancer develops. To this end, we have been developing a transformed cell line, based on repeated *in vitro* passaging of primary keratinocytes transfected with human papillomavirus, followed by treatment with the carcinogen, benzo(a)pyrene. We have used this cell line to screen for PTK inhibitors with differential effects on transformed keratinocytes versus primary keratinocytes (Ben-Bassat *et al.*, 1999, and unpublished data). We hope to "capture" and characterize the very earliest steps in carcinogenesis, even before the recognition of preneoplastic lesions. This knowledge will help us to find biomarkers for early detection and targets for timely intervention, as well as to develop economical *in vitro* screens for cancer drugs. So-called "raft" cultures of keratinocytes (papillomavirus-transformed or otherwise) attempt to model the three-dimensional properties of skin (Delvenne *et al.*, 2001; Flores *et al.*, 1999). Other groups have taken the approach of introducing known genetic alterations into primary cells and following their transformation into malignant cells (Elenbaas *et al.*, 2001; Milyavsky *et al.*, 2005). All of these studies rely on microarray and proteomic technologies to provide a global picture of the changes that occur in the cells on transformation. None of these models considers the roles of the surrounding stromal tissue, interstitial space, developing blood vessels, or the immune system in tumor development, although some of these issues can be addressed by coculture of more than one cell type.

B. *In Vivo* Models: Xenografts

The most accessible models for looking at candidate cancer drugs in the context of the whole animal are human cancer xenografts in immunocompromised mice. These xenografts are often subcutaneous, in which case growth of a solid tumor can be easily assessed. Intraperitoneal xenografts are also common. Injecting these types of xenografts does not require any particular surgical skills. It is easier to generate xenografts from cell lines than from primary tumor tissue, although the latter models are more closely

related to the human disease. Xenografts can be established using cells that have been genetically manipulated to express a specific oncogene, or appropriate cell lines known to express the target. When it is important to imitate the natural environment of the tumor, such as when studying angiogenic factors, an orthotopic (i.e., to the same tissue) xenograft may be preferred (Bibby, 2004; Killion *et al*., 1998). Orthotopic grafts are better models for metastatic cancer. Despite metabolic differences between mice and men, xenograft models are useful for preliminary pharmacological characterization. Nonetheless, many drug candidates still fail during clinical trials.

C. Transgenic Models

The lack of adequate cancer models is all the more serious when we consider that the earlier a tumor can be detected, the more effective is the treatment. In attempts to follow the earliest steps in tumorigenesis, transgenic mouse models have been established (Becher and Holland, 2006; Herzig and Christofori, 2002; Rosenberg and Bortner, 1998). These models are expensive and difficult to establish, and often do not faithfully mimic human disease. An advantage of transgenic models is that they can be generated in immunocompetent mice. Transgenic models are good for looking at the consequences of a known mutation, but they are biased in the sense that the initiating mutation of human cancer is usually not certain.

Transgenic mice can be reasonably good models for some inherited cancer predisposition syndromes. For instance, mice carrying heterozygous *Apc* mutations develop polyps of the small intestine, whereas the same mutations in humans generally lead to polyps in the colon, a condition known as familial adenomatous polyposis (reviewed in Taketo, 2006). On the other hand, unlike humans, mouse *Rb1* heterozygous knockouts do not develop retinoblastoma, unless they carry an additional mutation in the *p107* tumor suppressor gene (Robanus-Maandag *et al*., 1998). Newer mouse models of retinoblastoma involving orthotopic xenografts of human retinoblastoma cells directly into the eyes of newborn rats have been used to design chemotherapeutic regimens (Laurie *et al*., 2005).

Germ-line-generated transgenic mice express the engineered mutation in all cells of the body. It is often supposed that transgenic mice in which the engineered gene is expressed inducibly in a tissue-specific manner are more accurate models of cancer. Yet, cancers develop following transformation of a single cell, not of all the cells in a given tissue. Therefore, even the inducible, tissue-specific transgenic models are far from faithful mimics of natural cancer development. Indeed, in this respect, xenograft models more closely imitate the natural disease.

The choice of preclinical model strongly influences the results obtained. A pertinent example is the history of farnesyl transferase inhibitors (Downward, 2003). The small G-protein, Ras, is an important regulator of cell growth. Activating mutations in the three *RAS* family members, *HRAS*, *KRAS*, and *NRAS*, are associated with a significant fraction of tumors. Because Ras is posttranslationally farnesylated, it was hoped that farnesyl transferase inhibitors would be effective against tumors with activating *RAS* mutations. In order to validate this approach, transgenic mice overexpressing oncogenic *v-Hras* were brought into play. These mice developed palpable tumors, which regressed on administration of a farnesyl transferase inhibitor (Kohl *et al.*, 1995). Unfortunately, in human cancer, *Hras* mutations are the exception rather than the rule; mutations in *Kras* are much more common. Unlike HRas, which must be farnesylated to be active, KRas can function in the absence of farnesylation, as it is also subject to another posttranslational modification, geranylgeranylation. In retrospect, therefore, it is not surprising that farnesyl transferase inhibitors have failed in the clinic.

D. Clinical Trials

In the final analysis, and given the limitations of the available preclinical models, the only way to evaluate a drug and determine the best regimen for drug delivery is to test it on human patients. The advent of targeted drugs also affects how clinical trials are designed. Although the principal question is whether a drug improves survival of cancer patients without unacceptable side effects, targeted therapy offers the opportunity to validate target inhibition long before survival data are available. Furthermore, if and when resistance emerges, we would like to know whether the target molecule has been itself mutated or whether it has been bypassed (e.g., by activation of a compensatory pathway), in order to design strategies to prevent resistance.

V. HOW SUCCESSFUL IS SIGNAL TRANSDUCTION THERAPY IN THE CLINIC?

A number of signal transduction inhibitors have been approved for clinical use and many more are in advanced clinical trials. In this chapter, we use some of the best-studied inhibitors (Table I) to illustrate the problems and successes of signal transduction therapy to date.

Table I Signal Transduction Inhibitors Aimed at Cancer in the Clinic

Agent	Molecular target	Targeted tumor
Small molecules		
Gleevec/STI 571/ imatinib	BCR-ABL Kit, PDGFR PDGFR	Chronic myeloid leukemia Gastrointestinal stromal tumor Chronic eosinophilic leukemia
Iressa/ZD1839/ gefitinib	EGFR	Non-small cell lung carcinoma, colon carcinoma, glioblastoma multiforme
Tarceva/OSI-774/ erlotinib	EGFR (ErbB1)	Non-small cell lung carcinoma, glioblastoma multiforme, renal cell carcinoma
Tykerb/GW572016/ Lapatinib	EGFR and ErbB2 (HER-2)	Breast cancer
Antibodies		
Herceptin/ trastuzumab	ErbB2 (HER-2)	Breast cancer
Erbitux	EGFR	Head and neck squamous cell carcinoma
Avastin/ bevacuzimab	VEGFR	Colon carcinoma, renal cell carcinoma

A. CML and Gleevec

The most dramatic success in the clinic has been achieved with Gleevec in the treatment of CML. This disease is unusual in that it is almost invariably associated with a translocation that leads to the formation of the *BCR-ABL* oncogene. The resulting kinase activates the proliferative Ras-MAPK and JAK-STAT pathways, as well as the anti-apoptotic PI3K-AKT and Bcl-2 pathways (Shet *et al.*, 2002). In early phase CML, the BCR-ABL kinase is the major survival element in the leukemic cells and its inhibition by Gleevec causes their demise. This is not the case for more advanced disease. Gleevec has a temporary effect on acute CML, with disease recurrence in a matter of months.

Gleevec does not eradicate CML. Complete molecular remission is rare, and relapse occurs if treatment is stopped (Cortes *et al.*, 2004; Higashi *et al.*, 2004). Therefore, patients are now kept on Gleevec indefinitely, unless resistance emerges. A number of mechanisms of resistance to Gleevec have been characterized (Table II), the most common being mutations in the ABL kinase domain (Gorre *et al.*, 2001; Roche-Lestienne and Preudhomme, 2003; Roche-Lestienne *et al.*, 2002). Other mechanisms include amplification of the *BCR-ABL* gene (Shah *et al.*, 2002) and the enhancement of BCR-ABL independent oncogenic pathways (Donato *et al.*, 2003; Ptasznik *et al.*, 2004). In the design of second generation drugs to overcome Gleevec

Table II Mechanisms of Resistance to Signal Transduction Inhibitors

Mechanism	Example	Resistance to	References
Mutation	Mutations in *BCR-ABL*, *kit*, and *PDGFR* kinase domains	Gleevec	Gorre *et al*., 2001; Schindler *et al*., 2000
	Mutations in *EGFR* kinase domain	Iressa and Tarceva	Pao *et al*., 2005
Gene amplification	*BCR-ABL* amplification	Gleevec	Gorre *et al*., 2001
Activation of alternative pathways	Activation of Src family signaling	Gleevec	Donato *et al*., 2003; Ptasznik *et al*., 2004
	Activation of ER pathway	Lapatinib	Xia *et al*., 2006

resistance, several factors need to be considered. If we consider Gleevec and BCR-ABL to be a paradigm for signal transduction inhibitors, we shall be able to apply the lessons we learn from the CML story to other cancers.

1. TARGETING THE ACTIVE KINASE CONFORMATION

A series of elegant experiments incorporating *in vitro* mutagenesis and screening for drug resistance, combined with crystal structure analysis, have been used to classify the mutations that can lead to Gleevec resistance. Most of the mutations that lead to Gleevec resistance affect binding of the drug, either by changing the drug-kinase contact residues or by preventing the kinase from adopting the specific inactive conformation to which Gleevec binds (Azam *et al*., 2003; Nagar *et al*., 2002; Shah *et al*., 2002). New drugs are being tested that bind both active and inactive forms of the kinase (Nagar *et al*., 2002; O'Hare *et al*., 2005; Shah *et al*., 2004; von Bubnoff *et al*., 2006). Dasatinib (BMS-354825) targets both ABL and Src kinases. Despite being less specific than Gleevec, dasatinib is much more potent than Gleevec, and is active against almost all of the clinically relevant *BCR-ABL* mutants, the exception being the T315I mutant. The crystal structure of dasatinib-bound ABL kinase suggests this drug can bind multiple states of the kinase, including the active conformation (Tokarski *et al*., 2006). Early clinical trials of dasatinib have been encouraging. Inhibitors that target the active conformation of an enzyme should be more potent and less toxic than inhibitors of the inactive conformation, because the active state is present only transiently in healthy cells, whereas it is the predominant form of the enzyme in the targeted cancer cells (Levitzki and Bohmer, 1998).

2. TARGETING THE SUBSTRATE-BINDING SITE

Most current small molecular weight PTK inhibitors, including Gleevec, are ATP mimics. Because the ATP-binding site is strongly conserved among kinases, ATP-competitive inhibitors tend to be poorly selective and to target several related kinases. Indeed, Gleevec itself inhibits not only BCR-ABL, but also the Kit and PDGFR kinases (Buchdunger *et al.*, 1995, 2000). For this reason Gleevec is used to treat gastrointestinal stromal tumors (GIST), which are dependent on activated Kit or, occasionally, PDGFRα (Hirota *et al.*, 1998, 2003; Rubin *et al.*, 2001). Some of the earliest PTK inhibitors were substrate-competitive (Anafi *et al.*, 1992; Shechter *et al.*, 1989; Yaish *et al.*, 1988). Recently, a substrate-competitive inhibitor of BCR-ABL, ON012380, has been described. This molecule is effective against all of the clinically relevant Gleevec-resistant mutants, including the T315I mutant, and synergizes well with Gleevec in preclinical models (Gumireddy *et al.*, 2005). Drug resistance is less likely to emerge during combination treatment using Gleevec and ON012380, as two separate mutations would be required.

3. MULTIPLE TARGETS

Although traditionally we have believed that the more specific an inhibitor the less likely it is to cause side effects, there are sometimes advantages to using inhibitors with more than one target. The commercial incentive is clear: Gleevec is used not only in the treatment of CML but also of GIST, as well as chronic myelomonocytic leukemia (CMML), which is sometimes associated with PDGFRβ activation (Apperley *et al.*, 2002; Golub *et al.*, 1994) and of chronic eosinophilic leukemia (CEL), which is occasionally associated with PDGFRα activation (Awada *et al.*, 2005). The issue also impinges on our understanding of how signal transduction therapy should work: it is the rare cancer that is dependent on one principle survival factor. Therefore, we need to inhibit several oncogenic pathways in order to destroy most tumors. A drug that targets multiple survival signals within the tumor effectively provides "combination" therapy in a single formulation. Resistance to Gleevec occasionally is caused by the compensatory activation of Src family kinases (Donato *et al.*, 2003; Ptasznik *et al.*, 2004); use of a dual Src-Abl inhibitor such as dasatinib should preclude this mode of resistance.

4. TUMOR STEM CELLS

Considerable evidence has accumulated that both hematopoietic (Hope *et al.*, 2004; Miyamoto *et al.*, 2000; Warner *et al.*, 2004) and solid tumors (Al-Hajj *et al.*, 2003; Kim *et al.*, 2005; Singh *et al.*, 2004) include a population

of self-renewing "cancer stem cells" that carry cancer specific markers. Thus, even if the bulk of a tumor is eradicated, the cancer stem cells may be capable of regenerating the tumor. Unfortunately, cancer stem cells tend to be refractory to current treatments. Several groups are profiling cancer stem cells using microarray technologies, in order to identify possible therapeutic targets (Almstrup *et al*., 2004; Georgantas *et al*., 2004).

Until the advent of Gleevec, CML was treated with interferon. *In vitro* studies show that CML stem cells are more sensitive to interferon than are differentiated CML cells, whereas the differentiated cells are more sensitive to Gleevec (Angstreich *et al*., 2005). The combination of interferon and Gleevec is now in clinical trials. Along a similar vein, in a murine myeloma tumor model, combined therapy with a JAK2 inhibitor and IL-12 was more effective than either agent alone (Burdelya *et al*., 2002). Unlike cytotoxic treatments, PTK inhibitors do not disarm the immune system. Thus, control of cancer might be achievable by using PTK inhibitors to reduce tumor load and cytokines to stimulate long-term immunity against the remaining tumor cells.

B. Inhibiting the EGFR Family

The EGFR family comprises four members: EGFR (ErbB1), ErbB2 (Her-2), and ErbB4 have catalytic activity; ErbB3 is catalytically inactive but retains ligand binding. These receptors are activated by ligand-induced dimerization in assorted homo- and heterodimeric forms. Overexpression and/or abnormal activation of EGFR family members is associated with a wide spectrum of solid tumors, including non-small cell lung carcinoma (NSCLC), breast cancer, pancreatic cancer, colon cancer, glioblastoma multiforme (GBM), and head and neck cancer (Citri and Yarden, 2006). EGFR was therefore an early candidate for signal transduction inhibition. Some of the first PTK inhibitors were directed against the EGFR and since then dozens of small molecule inhibitors have been reported (reviewed in Levitzki and Mishani, 2006). Two EGFR kinase inhibitors, Iressa (gefitinib/ZD 1839) and Tarceva (erlotinib/OSI-774), and an antibody against the EGFR, Erbitux (cetuximab), have already been approved for clinical use.

1. TARGETING TUMOR SURVIVAL FACTORS

EGFR is overexpressed in over 80% of NSCLC, so it was hoped that drugs like Iressa would have a major impact on treatment of the disease. But these hopes were shattered when only 10% of NSCLC patients responded to Iressa in clinical trials in the United States. It thus became clear that

overexpression of a purported oncogene does not suffice to predict tumor dependence on that oncogene.

The frequency of response to Iressa was higher among women, nonsmokers, and East Asians. Responsive tumors tend to have specific activating mutations in the EGFR kinase domain that lead to stimulation of the anti-apoptotic PI3K-PKB and JAK-STAT pathways (Lynch *et al.*, 2004; Paez *et al.*, 2004; Sordella *et al.*, 2004). Structural studies have shown that binding of Tarceva, which is similar to Iressa, requires the active conformation of EGFR. The EGFR-activating mutations force the receptor into its active conformation, rendering the molecule more sensitive to inhibitors such as Tarceva and Iressa (Zhang *et al.*, 2006). This is reminiscent of the situation with GIST, where activating mutations in Kit render the tumors more susceptible to Gleevec (Debiec-Rychter *et al.*, 2004; Heinrich *et al.*, 2003).

Recent studies have confirmed that these mutations convert EGFR into a strongly oncogenic form that can be an essential tumor survival factor, at least in mice. When mutant EGFR was inducibly expressed in mouse pneumocyte cells, lung adenocarcinomas developed. The tumors regressed on treatment with Tarceva or the anti-EGFR antibody, Erbitux, or when EGFR expression was stopped by withdrawal of doxycycline (Ji *et al.*, 2006; Politi *et al.*, 2006). For human NSCLC, there appear to be additional factors that affect the response to treatment with Iressa or Tarceva. These include strong overexpression of EGFR, coactivation and overexpression of the EGFR-related receptor ErbB2 (HER-2) (Cappuzzo *et al.*, 2005b), and activation of PKB (Cappuzzo *et al.*, 2005a).

Unfortunately, even patients who do respond to Iressa or Tarceva have a short reprieve as they quickly develop drug resistance (Table II), owing to secondary mutations in the *EGFR* gene (Pao *et al.*, 2005).

2. COMBINED THERAPY

A variation on the theme of "combination therapy" is to target the same signaling molecule with more than one therapeutic moiety. Thus, in preclinical studies, synergy or additivity occurred when EGFR was targeted by small molecule inhibitors together with monoclonal antibodies (Huang *et al.*, 2004; Matar *et al.*, 2004; Yoneda *et al.*, 1991). Cooperative behavior between PTK inhibitors and antibodies is dependent on the cell type in which it is examined, and may involve coinhibition of related EGFR-like heterodimers, such as EGFR-ErbB2. On the other hand, pancreatic carcinoma cells that expressed EGFR and ErbB3 were resistant to the monoclonal antibody Erbitux, apparently because the presence of ErbB3 prevented antibody-mediated internalization of EGFR (Arnoletti *et al.*, 2004; Lenferink *et al.*, 1998).

A more common approach to combination therapy is to target several different pathways that affect tumor growth. Avastin (bevacizumab; an antibody to VEGF) is approved for the treatment of patients with advanced colon carcinoma, who derive short-term benefit from the antibody. Advanced renal carcinoma is highly metastatic and appears to depend strongly on neovascularization. Thus, VEGF inhibitors provide a slight benefit to renal carcinoma patients, who generally have a very poor prognosis. The effectiveness of combining Tarceva and Avastin is being tested on several types of cancer. Results from patients with advanced metastatic renal carcinoma who were treated with a combination of Tarceva and Avastin are very encouraging (Hainsworth *et al*., 2005). Of 59 patients enrolled in a phase II trial, 87% had a clear clinical benefit: tumor load was reduced by at least 50% in 21% of patients, and the disease stabilized in a further 66%. At 18 months, 60% of patients were still alive. If these results hold up when the treatment is extended to large numbers of patients, they will be unprecedented for this type of cancer.

Advanced tumors tend to be desensitized to apoptotic signals and refractory to chemotherapy. Targeted inhibition of essential tumor survival factors can resensitize the tumor to cytotoxic chemotherapy as originally shown by Tsai *et al*. (1996). Thus, small molecule inhibitors often synergize with chemotherapeutic treatments. About a quarter of breast cancers overexpress ErbB2. These tumors are resistant to hormone therapy and, until recently, carried a poorer prognosis than hormone-responsive tumors. Inhibition of ErbB2 by small molecules or by the monoclonal antibody Herceptin (trastuzumab) is synergistic with certain forms of chemotherapy (Yeon and Pegram, 2005).

The additional factors, other than strong overexpression of ErbB2, that determine which breast cancer patients will respond to Herceptin are not clear. In any event, the benefit derived by patients with advanced disease is unfortunately slight. On the other hand, treatment by chemotherapy plus Herceptin was twice as effective in preventing the recurrence of ErbB2-overexpressing breast cancer in patients with early, localized disease as was chemotherapy alone (Piccart-Gebhart *et al*., 2005; Romond *et al*., 2005). Signal transduction inhibitors that have been found to give only slight benefit to advanced cancer patients may well be more effective when given to patients with earlier stage disease.

The synergistic effect of Herceptin with chemotherapy may be at least partly due to the fact that Herceptin reduces levels of DNA repair following radiation or cisplatin treatment, thus increasing cytotoxicity (Pietras *et al*., 1994, 1999). Herceptin is another good example of a multiple inhibitor. Not only does Herceptin inhibit ErbB2 signaling, it also inhibits angiogenesis, which is stimulated by ErbB2 (Izumi *et al*., 2002). Some of its power may also be due to its ability to invoke antibody-dependent cell-mediated

cytotoxicity. Another inhibitor with multiple targets is Tykerb (lapatinib/GW572016). This dual EGFR-ErbB2 inhibitor is in advanced clinical trials, as a single agent and in combination with chemotherapy. Phase III trials showed delay in disease progression among women with advanced, Herceptin-resistant ErbB2-positive breast cancer (Geyer *et al.*, 2006). *In vitro* studies suggest that one mechanism of acquiring resistance to Tykerb is increased signaling from the ER. These experiments point the way to clinical testing of Tykerb together with aromatase inhibitors for patients with ErbB2-positive, ER-positive tumors (Xia *et al.*, 2006).

Combination regimens that look promising in preclinical models can fail in the clinic. Combining anti-EGFR inhibitors with cisplatin treatment was synergistic in combating EGFR-overexpressing glioblastoma in nude mice (Nagane *et al.*, 1998, 2001). A similar combination was not effective in clinical trials to treat NSCLC (Tamura and Fukuoka, 2005), but it is problematic to attempt to extrapolate from a glioma xenograft to human lung cancer.

Extremely encouraging results were released from a clinical trial testing the combination of Erbitux and radiation to treat head and neck cancer (Bonner *et al.*, 2006). Earlier *in vitro* and xenograft studies on head and neck squamous cell carcinoma showed that Erbitux enhanced sensitivity to radiation. Like Herceptin, Erbitux appears to inhibit DNA repair and angiogenesis following radiation (Huang and Harari, 2000). A phase III clinical trial showed significant tumor shrinkage, delay in progression and increased survival when Erbitux was added to the standard radiation regimen (Bonner *et al.*, 2006).

For most tumors, targeted signal transduction inhibitors will not replace the traditional cytotoxic chemotherapy and radiotherapy. Rather, it will be necessary to find the optimal combinations of targeted therapy and cytotoxic therapy for each tumor type.

VI. USING PK RECEPTORS AS HOMING MOLECULES FOR CANCER THERAPY

There is no simple way to predict whether a given RPTK functions as an essential tumor survival factor, but one can still use the RPTK as a "homing device" for signal transduction or other therapy. Our laboratory is developing this idea for the treatment of glioblastoma, as well as other cancers. One approach involves using EGF bound to a vector carrying synthetic dsRNA to introduce the dsRNA into EGF-overexpressing glioma cells. This activates the pro-apoptotic dsRNA-dependent protein kinase, PKR, leading to death of

the glioma cells. Even more encouraging, the dying cells release cytokines that lead to a "bystander" effect so that neighboring glioma cells that do not overexpress EGFR also die (Shir *et al.*, 2006). This is important because most tumors are heterogeneous; by invoking a bystander effect, even nontargeted tumor cells can be induced to die (Shir and Levitzki, 2001). Normal cells remain unharmed because they are more robust and resist stress.

Other strategies for directing nonspecific therapies to cancer cells include the use of antibodies coupled to toxic or radioactive molecules (Kaminski *et al.*, 2005; Lemieux and Coiffier, 2005; Pastan, 2003). For these strategies to work, it is sufficient to identify a unique or strongly overexpressed receptor molecule on cancer cells; this receptor does not have to play any survival role in the life of the cell.

VII. CONCLUSIONS

The impact of signal transduction therapy on cancer statistics has been miniscule so far. Cancer is the second leading cause of death in the United States, and the probability of contracting cancer at some point in one's lifetime is on the order of 40%. Billions of research dollars have been spent in combating this deathly disease. Recently, the incidence of cancer has shown signs of stabilizing and death rates have begun to decrease, at a rate of about 1% per year (Jemal *et al.*, 2005). Most of this decrease can probably be attributed to earlier detection and intervention.

A cold cost-benefit analysis might lead to the conclusion that we are barking up the wrong tree. Part of this disappointment is inevitable: clinical trials are performed on patients for whom other therapies have failed. By definition, these are the patients with the most advanced and recalcitrant disease. Just as Gleevec is much more effective against early chronic CML than against advanced blast crisis disease, and as Herceptin significantly reduces the incidence of recurrence of early stage breast cancer while delaying progression of advanced breast cancer by only a few months, other signal transduction inhibitors will also be more effective at combating early than late disease. As more targeted drugs become licensed and can be used to treat early cancer, we anticipate that signal transduction therapy will begin to make its mark.

Targeted cancer therapy, as its name suggests, needs to be applied in a specific manner. Slowly, we are learning which cancers respond to which therapies, and which markers to look for to assist in choosing appropriate drugs. Although a trial of Avastin (together with chemotherapy) on pancreatic cancer recently failed, results so far for renal carcinoma (together with Tarceva) are very encouraging. Furthermore, with the exception of some

hematological neoplasms, most cancers, even of the same tissue, include different forms of the disease. Treatment of breast cancer already takes into account whether the tumor is hormone responsive or not, and whether ErbB2 is overexpressed on the tumor and to what extent. "Personalized" treatment, using genomic or proteomic techniques to determine which are the most susceptible molecular targets in a specific patient, is still a futuristic idea, but is no longer a wild science fiction fantasy.

When interpreting preclinical data, we must bear in mind that a model is just that: it should be as close as we can reasonably get—given constraints of time and money—to the human disease, but it is very far from the real thing. We have to clarify to ourselves which questions our model can answer and where the model may serve only to reinforce our own preconceptions.

Recent experience shows that using signal transduction inhibitors as monotherapy is unlikely to give pronounced benefit in most cancers: CML is the exception, rather than the rule, and even for CML it is clear that Gleevec is not the whole answer, as some patients are resistant and a significant proportion of responsive patients eventually develop resistance. Combination therapies are more effective than single therapies. Combining signal transduction inhibitors against several pathways affecting growth and survival may defeat certain cancers, even when a major survival factor is not apparent. Furthermore, combining therapies is the most effective method to reduce the emergence of resistance. Signal transduction therapy can sensitize cancers to chemotherapy and radiotherapy such that doses can be reduced and toxic side effects minimized. A major challenge is posed by cancer stem cells, which are believed to be responsible for recurrent disease and metastases. Signal transduction therapy may also be used in conjunction with immunotherapy to provide longer term protection against recurrence. But the specific combinations and treatment regimens need to be worked out, and at present this can only be accomplished using real human patients, in clinical trials.

REFERENCES

Al-Hajj, M., Wicha, M. S., Benito-Hernandez, A., Morrison, S. J., and Clarke, M. F. (2003). Prospective identification of tumorigenic breast cancer cells. *Proc. Natl. Acad. Sci. USA* **100,** 3983–3988.

Almstrup, K., Hoei-Hansen, C. E., Wirkner, U., Blake, J., Schwager, C., Ansorge, W., Nielsen, J. E., Skakkebaek, N. E., Rajpert-De Meyts, E., and Leffers, H. (2004). Embryonic stem cell-like features of testicular carcinoma *in situ* revealed by genome-wide gene expression profiling. *Cancer Res.* **64,** 4736–4743.

Anafi, M., Gazit, A., Gilon, C., Ben-Neriah, Y., and Levitzki, A. (1992). Selective interactions of transforming and normal abl proteins with ATP, tyrosine-copolymer substrates, and tyrphostins. *J. Biol. Chem.* **267,** 4518–4523.

Angstreich, G. R., Matsui, W., Huff, C. A., Vala, M. S., Barber, J., Hawkins, A. L., Griffin, C. A., Smith, B. D., and Jones, R. J. (2005). Effects of imatinib and interferon on primitive chronic myeloid leukaemia progenitors. *Br. J. Haematol.* **130,** 373–381.

Apperley, J. F., Gardembas, M., Melo, J. V., Russell-Jones, R., Bain, B. J., Baxter, E. J., Chase, A., Chessells, J. M., Colombat, M., Dearden, C. E., Dimitrijevic, S., Mahon, F. X., *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,** 481–487.

Arnoletti, J. P., Buchsbaum, D. J., Huang, Z.-q., Hawkins, A. E., Khazaeli, M. B., Kraus, M. H., and Vickers, S. M. (2004). Mechanisms of resistance to Erbitux (anti-epidermal growth factor receptor) combination therapy in pancreatic adenocarcinoma cells. *J. Gastrointest. Surg.* **8,** 960–970.

Awada, A., Hendlisz, A., Gil, T., Bartholomeus, S., Mano, M., de Valeriola, D., Strumberg, D., Brendel, E., Haase, C. G., Schwartz, B., and Piccart, M. (2005). Phase I safety and pharmacokinetics of BAY 43-9006 administered for 21 days on/7 days off in patients with advanced, refractory solid tumours. *Br. J. Cancer* **92,** 1855–1861.

Azam, M., Latek, R. R., and Daley, G. Q. (2003). Mechanisms of autoinhibition and STI-571/imatinib resistance revealed by mutagenesis of BCR-ABL. *Cell* **112,** 831–843.

Banai, S., Gertz, S. D., Gavish, L., Chorny, M., Perez, L. S., Lazarovichi, G., Ianculuvich, M., Hoffmann, M., Orlowski, M., Golomb, G., and Levitzki, A. (2004). Tyrphostin AGL-2043 eluting stent reduces neointima formation in porcine coronary arteries. *Cardiovasc. Res.* **64,** 165–171.

Becher, O. J., and Holland, E. C. (2006). Genetically engineered models have advantages over xenografts for preclinical studies. *Cancer Res.* **66,** 3355–3358; discussion 3358–3359.

Ben-Bassat, H., Rosenbaum-Mitrani, S., Hartzstark, Z., Levitzki, R., Chaouat, M., Shlomai, Z., Klein, B. Y., Kleinberger-Doron, N., Gazit, A., Tsvieli, R., and Levitzki, A. (1999). Tyrphostins that suppress the growth of human papilloma virus 16-immortalized human keratinocytes. *J. Pharmacol. Exp. Ther.* **290,** 1442–1457.

Bibby, M. C. (2004). Orthotopic models of cancer for preclinical drug evaluation: Advantages and disadvantages. *Eur. J. Cancer* **40,** 852–857.

Bonner, J. A., Harari, P. M., Giralt, J., Azarnia, N., Shin, D. M., Cohen, R. B., Jones, C. U., Sur, R., Raben, D., Jassem, J., Ove, R., Kies, M. S., *et al.* (2006). Radiotherapy plus cetuximab for squamous-cell carcinoma of the head and neck 10.1056/NEJMoa053422. *N. Engl. J. Med.* **354,** 567–578.

Buchdunger, E., Zimmermann, J., Mett, H., Meyer, T., Muller, M., Regenass, U., and Lydon, N. B. (1995). Selective inhibition of the platelet-derived growth factor signal transduction pathway by a protein-tyrosine kinase inhibitor of the 2-phenylaminopyrimidine class. *Proc. Natl. Acad. Sci. USA* **92,** 2558–2562.

Buchdunger, E., Cioffi, C. L., Law, N., Stover, D., Ohno-Jones, S., Druker, B. J., and Lydon, N. B. (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,** 139–145.

Burdelya, L., Catlett-Falcone, R., Levitzki, A., Cheng, F., Mora, L. B., Sotomayor, E., Coppola, D., Sun, J., Sebti, S., Dalton, W. S., Jove, R., and Yu, H. (2002). Combination therapy with AG-490 and interleukin 12 achieves greater antitumor effects than either agent alone. *Mol. Cancer Ther.* **1,** 893–899.

Cao, C., Shinohara, E. T., Subhawong, T. K., Geng, L., Woon Kim, K., Albert, J. M., Hallahan, D. E., Lu, B., Coll-Mulet, L., Iglesias-Serret, D., Santidrian, A. F., Cosialls, A. M., *et al.* (2006). Radiosensitization of lung cancer by nutlin, an inhibitor of murine double minute 2 MDM2 antagonists activate p53 and synergize with genotoxic drugs in B-cell chronic lymphocytic leukemia cells. *Mol. Cancer Ther.* **5,** 411–417.

Cappuzzo, F., Hirsch, F. R., Rossi, E., Bartolini, S., Ceresoli, G. L., Bemis, L., Haney, J., Witta, S., Danenberg, K., Domenichini, I., Ludovini, V., Magrini, E., *et al.* (2005a). Epidermal growth factor receptor gene and protein and gefitinib sensitivity in non-small-cell lung cancer. *J. Natl. Cancer Inst.* **97,** 643–655.

Cappuzzo, F., Varella-Garcia, M., Shigematsu, H., Domenichini, I., Bartolini, S., Ceresoli, G. L., Rossi, E., Ludovini, V., Gregorc, V., Toschi, L., Franklin, W. A., Crino, L., *et al.* (2005b). Increased HER2 gene copy number is associated with response to gefitinib therapy in epidermal growth factor receptor-positive non-small-cell lung cancer patients epidermal growth factor receptor gene and protein and gefitinib sensitivity in non-small-cell lung cancer. *J. Clin. Oncol.* **23,** 5007–5018.

Citri, A., and Yarden, Y. (2006). EGF-ERBB signalling: Towards the systems level. *Nat. Rev. Mol. Cell Biol.* **7,** 505–516.

Coll-Mulet, L., Iglesias-Serret, D., Santidrian, A. F., Cosialls, A. M., de Frias, M., Castano, E., Campas, C., Barragan, M., de Sevilla, A. F., Domingo, A., Vassilev, L. T., Pons, G., *et al.* (2006). MDM2 antagonists activate p53 and synergize with genotoxic drugs in B-cell chronic lymphocytic leukemia cells. *Blood* **107,** 4109–4114.

Cortes, J., O'Brien, S., and Kantarjian, H. (2004). Discontinuation of imatinib therapy after achieving a molecular response. *Blood* **104,** 2204–2205.

De Keersmaecker, K., and Cools, J. (2006). Chronic myeloproliferative disorders: A tyrosine kinase tale. *Leukemia* **20,** 200–205.

Debiec-Rychter, M., Dumez, H., Judson, I., Wasag, B., Verweij, J., Brown, M., Dimitrijevic, S., Sciot, R., Stul, M., Vranck, H., Scurr, M., Hagemeijer, A., *et al.* (2004). Use of c-KIT/PDGFRA mutational analysis to predict the clinical response to imatinib in patients with advanced gastrointestinal stromal tumours entered on phase I and II studies of the EORTC Soft Tissue and Bone Sarcoma Group. *Eur. J. Cancer* **40,** 689–695.

Delvenne, P., Hubert, P., Jacobs, N., Giannini, S. L., Havard, L., Renard, I., Saboulard, D., and Boniver, J. (2001). The organotypic culture of HPV-transformed keratinocytes: An effective *in vitro* model for the development of new immunotherapeutic approaches for mucosal (pre) neoplastic lesions. *Vaccine* **19,** 2557–2564.

Donato, N. J., Wu, J. Y., Stapley, J., Gallick, G., Lin, H., Arlinghaus, R., and Talpaz, M. (2003). BCR-ABL independence and LYN kinase overexpression in chronic myelogenous leukemia cells selected for resistance to STI571. *Blood* **101,** 690–698.

Downward, J. (2003). Targeting ras signalling pathways in cancer therapy. *Nat. Rev. Cancer* **3,** 11–22.

Elenbaas, B., Spirio, L., Koerner, F., Fleming, M. D., Zimonjic, D. B., Donaher, J. L., Popescu, N. C., Hahn, W. C., and Weinberg, R. A. (2001). Human breast cancer cells generated by oncogenic transformation of primary mammary epithelial cells. *Genes Dev.* **15,** 50–65.

Fiebig, H. H., Maier, A., and Burger, A. M. (2004). Clonogenic assay with established human tumour xenografts: Correlation of *in vitro* to *in vivo* activity as a basis for anticancer drug discovery. *Eur. J. Cancer* **40,** 802–820.

Fisher, B., Costantino, J. P., Wickerham, D. L., Redmond, C. K., Kavanah, M., Cronin, W. M., Vogel, V., Robidoux, A., Dimitrov, N., Atkins, J., Daly, M., Wieand, S., *et al.* (1998). Tamoxifen for prevention of breast cancer: Report of the national surgical adjuvant breast and bowel project P-1 study. *J. Natl. Cancer Inst.* **90,** 1371–1388.

Flores, E. R., Allen-Hoffmann, B. L., Lee, D., Sattler, C. A., and Lambert, P. F. (1999). Establishment of the human papillomavirus type 16 (HPV-16) life cycle in an immortalized human foreskin keratinocyte cell line. *Virology* **262,** 344–354.

Georgantas, R. W., III, Tanadve, V., Malehorn, M., Heimfeld, S., Chen, C., Carr, L., Martinez-Murillo, F., Riggins, G., Kowalski, J., and Civin, C. I. (2004). Microarray and

serial analysis of gene expression analyses identify known and novel transcripts overexpressed in hematopoietic stem cells. *Cancer Res.* **64,** 4434–4441.
Geyer, C., Cameron, D., and Lindquist, D. (2006). *In* "42nd Annual Meeting of the American Society of Clinical Oncology." Atlanta, Georgia, USA.
Golub, T. R., Barker, G. F., Lovett, M., and Gilliland, D. G. (1994). Fusion of PDGF receptor beta to a novel ets-like gene, tel, in chronic myelomonocytic leukemia with t(5;12) chromosomal translocation. *Cell* **77,** 307–316.
Gorre, M. E., Mohammed, M., Ellwood, K., Hsu, N., Paquette, R., Rao, P. N., and Sawyers, C. L. (2001). Clinical resistance to STI-571 cancer therapy caused by BCR-ABL gene mutation or amplification. *Science* **293,** 876–880.
Gumireddy, K., Baker, S. J., Cosenza, S. C., John, P., Kang, A. D., Robell, K. A., Reddy, M. V., and Reddy, E. P. (2005). A non-ATP-competitive inhibitor of BCR-ABL overrides imatinib resistance. *Proc. Natl. Acad. Sci. USA* **102,** 1992–1997.
Hainsworth, J. D., Sosman, J. A., Spigel, D. R., Edwards, D. L., Baughman, C., and Greco, A. (2005). Treatment of metastatic renal cell carcinoma with a combination of bevacizumab and erlotinib. *J. Clin. Oncol.* **23,** 7889–7896.
Heinrich, M. C., Corless, C. L., Demetri, G. D., Blanke, C. D., von Mehren, M., Joensuu, H., McGreevey, L. S., Chen, C. J., Van den Abbeele, A. D., Druker, B. J., Kiese, B., Eisenberg, B., *et al.* (2003). Kinase mutations and imatinib response in patients with metastatic gastrointestinal stromal tumor. *J. Clin. Oncol.* **21,** 4342–4349.
Herzig, M., and Christofori, G. (2002). Recent advances in cancer research: Mouse models of tumorigenesis. *Biochim. Biophys. Acta* **1602,** 97–113.
Higashi, T., Tsukada, J., Kato, C., Iwashige, A., Mizobe, T., Machida, S., Morimoto, H., Ogawa, R., Toda, Y., and Tanaka, Y. (2004). Imatinib mesylate-sensitive blast crisis immediately after discontinuation of imatinib mesylate therapy in chronic myelogenous leukemia: Report of two cases. *Am. J. Hematol.* **76,** 275–278.
Hirota, S., Isozaki, K., Moriyama, Y., Hashimoto, K., Nishida, T., Ishiguro, S., Kawano, K., Hanada, M., Kurata, A., Takeda, M., Muhammad Tunio, G., Matsuzawa, Y., *et al.* (1998). Gain-of-function mutations of c-kit in human gastrointestinal stromal tumors. *Science* **279,** 577–580.
Hirota, S., Ohashi, A., Nishida, T., Isozaki, K., Kinoshita, K., Shinomura, Y., and Kitamura, Y. (2003). Gain-of-function mutations of platelet-derived growth factor receptor alpha gene in gastrointestinal stromal tumors. *Gastroenterology* **125,** 660–667.
Hope, K. J., Jin, L., and Dick, J. E. (2004). Acute myeloid leukemia originates from a hierarchy of leukemic stem cell classes that differ in self-renewal capacity. *Nat. Immunol.* **5,** 738–743.
Huang, S., Armstrong, E. A., Benavente, S., Chinnaiyan, P., and Harari, P. M. (2004). Dual-agent molecular targeting of the epidermal growth factor receptor (EGFR): Combining anti-EGFR antibody with tyrosine kinase inhibitor. *Cancer Res.* **64,** 5355–5362.
Huang, S.-M., and Harari, P. M. (2000). Modulation of radiation response after epidermal growth factor receptor blockade in squamous cell carcinomas: Inhibition of damage repair, cell cycle kinetics, and tumor angiogenesis. *Clin. Cancer Res.* **6,** 2166–2174.
Hurwitz, H., Fehrenbacher, L., Novotny, W., Cartwright, T., Hainsworth, J., Heim, W., Berlin, J., Baron, A., Griffing, S., Holmgren, E., Ferrara, N., Fyfe, G., *et al.* (2004). Bevacizumab plus irinotecan, fluorouracil, and leucovorin for metastatic colorectal cancer. *N. Engl. J. Med.* **350,** 2335–2342.
Izumi, Y., Xu, L., di Tomaso, E., Fukumura, D., and Jain, R. K. (2002). Tumour biology: Herceptin acts as an anti-angiogenic cocktail. *Nature* **416,** 279–280.
Jemal, A., Murray, T., Ward, E., Samuels, A., Tiwari, R. C., Ghafoor, A., Feuer, E. J., and Thun, M. J. (2005). Cancer statistics, 2005. *CA Cancer J. Clin.* **55,** 10–30.

Ji, H., Li, D., Chen, L., Shimamura, T., Kobayashi, S., McNamara, K., Mahmood, U., Mitchell, A., Sun, Y., and Al-Hashem, R. (2006). The impact of human EGFR kinase domain mutations on lung tumorigenesis and *in vivo* sensitivity to EGFR-targeted therapies. *Cancer Cell* **9,** 485–495.

Johnson, J. I., Decker, S., Zaharevitz, D., Rubinstein, L. V., Venditti, J. M., Schepartz, S., Kalyandrug, S., Christian, M., Arbuck, S., Hollingshead, M., and Sausville, E. A. (2001). Relationships between drug activity in NCI preclinical *in vitro* and *in vivo* models and early clinical trials. *Br. J. Cancer* **84,** 1424–1431.

Kaminski, M. S., Tuck, M., Estes, J., Kolstad, A., Ross, C. W., Zasadny, K., Regan, D., Kison, P., Fisher, S., Kroll, S., and Wahl, R. L. (2005). 131I-tositumomab therapy as initial treatment for follicular lymphoma. *N. Engl. J. Med.* **352,** 441–449.

Killion, J. J., Radinsky, R., and Fidler, I. J. (1998). Orthotopic models are necessary to predict therapy of transplantable tumors in mice. *Cancer Metastasis Rev.* **17,** 279–284.

Kim, C. F., Jackson, E. L., Woolfenden, A. E., Lawrence, S., Babar, I., Vogel, S., Crowley, D., Bronson, R. T., and Jacks, T. (2005). Identification of bronchioalveolar stem cells in normal lung and lung cancer. *Cell* **121,** 823–835.

Kohl, N. E., Omer, C. A., Conner, M. W., Anthony, N. J., Davide, J. P., deSolms, S. J., Giuliani, E. A., Gomez, R. P., Graham, S. L., Hamilton, K., Handt, L. K., Hartman, G. D., *et al.* (1995). Inhibition of farnesyltransferase induces regression of mammary and salivary carcinomas in ras transgenic mice. *Nat. Med.* **1,** 792–797.

Kong, D., Park, E. J., Stephen, A. G., Calvani, M., Cardellina, J. H., Monks, A., Fisher, R. J., Shoemaker, R. H., and Melillo, G. (2005). Echinomycin, a small-molecule inhibitor of hypoxia-inducible factor-1 DNA-binding activity. *Cancer Res.* **65,** 9047–9055.

Kung, A. L., Zabludoff, S. D., France, D. S., Freedman, S. J., Tanner, E. A., Vieira, A., Cornell-Kennon, S., Lee, J., Wang, B., Wang, J., Memmert, K., Naegeli, H. U., *et al.* (2004). Small molecule blockade of transcriptional coactivation of the hypoxia-inducible factor pathway. *Cancer Cell* **6,** 33–43.

Lane, D. P. (1992). Cancer. p53, guardian of the genome. *Nature* **358,** 15–16.

Laurie, N. A., Gray, J. K., Zhang, J., Leggas, M., Relling, M., Egorin, M., Stewart, C., and Dyer, M. A. (2005). Topotecan combination chemotherapy in two new rodent models of retinoblastoma. *Clin. Cancer Res.* **11,** 7569–7578.

Lemieux, B., and Coiffier, B. (2005). Radio-immunotherapy in low-grade non-Hodgkin's lymphoma. *Best. Pract. Res. Clin. Haematol.* **18,** 81–95.

Lenferink, A. E., Pinkas-Kramarski, R., van de Poll, M. L., van Vugt, M. J., Klapper, L. N., Tzahar, E., Waterman, H., Sela, M., van Zoelen, E. J., and Yarden, Y. (1998). Differential endocytic routing of homo- and hetero-dimeric ErbB tyrosine kinases confers signaling superiority to receptor heterodimers. *EMBO J.* **17,** 3385–3397.

Levitzki, A., and Bohmer, F. D. (1998). Altered efficacy and selectivity of tyrosine kinase inhibitors of the activated states of protein tyrosine kinases. *Anticancer Drug Des.* **13,** 731–734.

Levitzki, A., and Mishani, E. (2006). Tyrphostins and other tyrosine kinase inhibitors. *Annu. Rev. Biochem.* **75,** 93–109.

Luo, J., Manning, B. D., and Cantley, L. C. (2003). Targeting the PI3K-Akt pathway in human cancer: Rationale and promise. *Cancer Cell* **4,** 257–262.

Lynch, T. J., Bell, D. W., Sordella, R., Gurubhagavatula, S., Okimoto, R. A., Brannigan, B. W., Harris, P. L., Haserlat, S. M., Supko, J. G., Haluska, F. G., Louis, D. N., Christiani, D. C., *et al.* (2004). Activating mutations in the epidermal growth factor receptor underlying responsiveness of non-small-cell lung cancer to gefitinib. *N. Engl. J. Med.* **350,** 2129–2139.

Matar, P., Rojo, F., Cassia, R., Moreno-Bueno, G., Di Cosimo, S., Tabernero, J., Guzman, M., Rodriguez, S., Arribas, J., Palacios, J., and Baselga, J. (2004). Combined epidermal growth

factor receptor targeting with the tyrosine kinase inhibitor gefitinib (ZD1839) and the monoclonal antibody cetuximab (IMC-C225): Superiority over single-agent receptor targeting. *Clin. Cancer Res.* **10,** 6487–6501.

Milyavsky, M., Tabach, Y., Shats, I., Erez, N., Cohen, Y., Tang, X., Kalis, M., Kogan, I., Buganim, Y., Goldfinger, N., Ginsberg, D., Harris, C. C., *et al.* (2005). Transcriptional programs following genetic alterations in p53, INK4A, and H-Ras genes along defined stages of malignant transformation. *Cancer Res.* **65,** 4530–4543.

Miyamoto, T., Weissman, I. L., and Akashi, K. (2000). AML1/ETO-expressing nonleukemic stem cells in acute myelogenous leukemia with 8; 21 chromosomal translocation. *Proc. Natl. Acad. Sci. USA* **97,** 7521–7526.

Nagane, M., Levitzki, A., Gazit, A., Cavenee, W. K., and Huang, H. J. (1998). Drug resistance of human glioblastoma cells conferred by a tumor-specific mutant epidermal growth factor receptor through modulation of Bcl-XL and caspase-3-like proteases. *Proc. Natl. Acad. Sci. USA* **95,** 5724–5729.

Nagane, M., Narita, Y., Mishima, K., Levitzki, A., Burgess, A. W., Cavenee, W. K., and Huang, H. J. (2001). Human glioblastoma xenografts overexpressing a tumor-specific mutant epidermal growth factor receptor sensitized to cisplatin by the AG1478 tyrosine kinase inhibitor. *J. Neurosurg.* **95,** 472–479.

Nagar, B., Bornmann, W. G., Pellicena, P., Schindler, T., Veach, D. R., Miller, W. T., Clarkson, B., and Kuriyan, J. (2002). Crystal structures of the kinase domain of c-Abl in complex with the small molecule inhibitors PD173955 and imatinib (STI-571). *Cancer Res.* **62,** 4236–4243.

O'Hare, T., Walters, D. K., Stoffregen, E. P., Jia, T., Manley, P. W., Mestan, J., Cowan-Jacob, S. W., Lee, F. Y., Heinrich, M. C., Deininger, M. W., and Druker, B. J. (2005). *In vitro* activity of Bcr-Abl inhibitors AMN107 and BMS-354825 against clinically relevant imatinib-resistant Abl kinase domain mutants. *Cancer Res.* **65,** 4500–4505.

Paez, J. G., Janne, P. A., Lee, J. C., Tracy, S., Greulich, H., Gabriel, S., Herman, P., Kaye, F. J., Lindeman, N., Boggon, T. J., Naoki, K., Sasaki, H., *et al.* (2004). EGFR mutations in lung cancer: Correlation with clinical response to gefitinib therapy. *Science* **304,** 1497–1500.

Pao, W., Miller, V. A., Politi, K. A., Riely, G. J., Somwar, R., Zakowski, M. F., Kris, M. G., and Varmus, H. (2005). Acquired resistance of lung adenocarcinomas to gefitinib or erlotinib is associated with a second mutation in the EGFR kinase domain. *PLoS Med.* **2,** e73.

Pastan, I. (2003). Immunotoxins containing Pseudomonas exotoxin A: A short history. *Cancer Immunol. Immunother.* **52,** 338–341.

Piccart-Gebhart, M. J., Procter, M., Leyland-Jones, B., Goldhirsch, A., Untch, M., Smith, I., Gianni, L., Baselga, J., Bell, R., Jackisch, C., Cameron, D., Dowsett, M., *et al.* (2005). Trastuzumab after adjuvant chemotherapy in HER2-positive breast cancer. *N. Engl. J. Med.* **353,** 1659–1672.

Pietras, R. J., Fendly, B. M., Chazin, V. R., Pegram, M. D., Howell, S. B., and Slamon, D. J. (1994). Antibody to HER-2/neu receptor blocks DNA repair after cisplatin in human breast and ovarian cancer cells. *Oncogene* **9,** 1829–1838.

Pietras, R. J., Poen, J. C., Gallardo, D., Wongvipat, P. N., Lee, H. J., Slamon, D. J., Fendly, B. M., Chazin, V. R., Pegram, M. D., and Howell, S. B. (1999). Monoclonal antibody to HER-2/neureceptor modulates repair of radiation-induced DNA damage and enhances radiosensitivity of human breast cancer cells overexpressing this oncogene antibody to HER-2/neu receptor blocks DNA repair after cisplatin in human breast and ovarian cancer cells. *Cancer Res.* **59,** 1347–1355.

Politi, K., Zakowski, M. F., Fan, P.-D., Schonfeld, E. A., Pao, W., and Varmus, H. E. (2006). Lung adenocarcinomas induced in mice by mutant EGF receptors found in human lung

cancers respond to a tyrosine kinase inhibitor or to down-regulation of the receptors 10.1101/gad.1417406. *Genes Dev.* **20,** 1496–1510.

Powis, G., and Kirkpatrick, L. (2004). Hypoxia inducible factor-1alpha as a cancer drug target. *Mol. Cancer Ther.* **3,** 647–654.

Ptasznik, A., Nakata, Y., Kalota, A., Emerson, S. G., and Gewirtz, A. M. (2004). Short interfering RNA (siRNA) targeting the Lyn kinase induces apoptosis in primary, and drug-resistant, BCR-ABL1(+) leukemia cells. *Nat. Med.* **10,** 1187–1189.

Robanus-Maandag, E., Dekker, M., van der Valk, M., Carrozza, M. L., Jeanny, J. C., Dannenberg, J. H., Berns, A., and te Riele, H. (1998). p107 is a suppressor of retinoblastoma development in pRb-deficient mice. *Genes Dev.* **12,** 1599–1609.

Roche-Lestienne, C., and Preudhomme, C. (2003). Mutations in the ABL kinase domain pre-exist the onset of imatinib treatment. *Semin. Hematol.* **40,** 80–82.

Roche-Lestienne, C., Soenen-Cornu, V., Grardel-Duflos, N., Lai, J. L., Philippe, N., Facon, T., Fenaux, P., and Preudhomme, C. (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,** 1014–1018.

Romond, E. H., Perez, E. A., Bryant, J., Suman, V. J., Geyer, C. E., Jr., Davidson, N. E., Tan-Chiu, E., Martino, S., Paik, S., Kaufman, P. A., Swain, S. M., Pisansky, T. M., *et al.* (2005). Trastuzumab plus adjuvant chemotherapy for operable HER2-positive breast cancer. *N. Engl. J. Med.* **353,** 1673–1684.

Rosenberg, M. P., and Bortner, D. (1998). Why transgenic and knockout animal models should be used (for Drug efficacy studies in cancer). *Cancer Metastasis Rev.* **17,** 295–299.

Rubin, B. P., Singer, S., Tsao, C., Duensing, A., Lux, M. L., Ruiz, R., Hibbard, M. K., Chen, C. J., Xiao, S., Tuveson, D. A., Demetri, G. D., Fletcher, C. D., *et al.* (2001). KIT activation is a ubiquitous feature of gastrointestinal stromal tumors. *Cancer Res.* **61,** 8118–8121.

Schafer, A. I. (2006). Molecular basis of the diagnosis and treatment of polycythemia vera and essential thrombocythemia. *Blood* **107,** 4214–4222.

Schimmer, A. D., Welsh, K., Pinilla, C., Wang, Z., Krajewska, M., Bonneau, M. J., Pedersen, I. M., Kitada, S., Scott, F. L., Bailly-Maitre, B., Glinsky, G., Scudiero, D., *et al.* (2004). Small-molecule antagonists of apoptosis suppressor XIAP exhibit broad antitumor activity. *Cancer Cell* **5,** 25–35.

Schindler, T., Bornmann, W., Pellicena, P., Miller, W. T., Clarkson, B., and Kuriyan, J. (2000). Structural mechanism for STI-571 inhibition of abelson tyrosine kinase. *Science* **289,** 1938–1942.

Selivanova, G., Kawasaki, T., Ryabchenko, L., and Wiman, K. G. (1998). Reactivation of mutant p53: A new strategy for cancer therapy. *Semin. Cancer Biol.* **8,** 369–378.

Shah, N. P., Nicoll, J. M., Nagar, B., Gorre, M. E., Paquette, R. L., Kuriyan, J., and Sawyers, C. L. (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,** 117–125.

Shah, N. P., Tran, C., Lee, F. Y., Chen, P., Norris, D., and Sawyers, C. L. (2004). Overriding imatinib resistance with a novel ABL kinase inhibitor. *Science* **305,** 399–401.

Shechter, Y., Yaish, P., Chorev, M., Gilon, C., Braun, S., and Levitzki, A. (1989). Inhibition of insulin-dependent lipogenesis and anti-lipolysis by protein tyrosine kinase inhibitors. *EMBO J.* **8,** 1671–1676.

Shet, A. S., Jahagirdar, B. N., and Verfaillie, C. M. (2002). Chronic myelogenous leukemia: Mechanisms underlying disease progression. *Leukemia* **16,** 1402–1411.

Shir, A., and Levitzki, A. (2001). Commentary: Gene therapy for glioblastoma: Future perspective for delivery systems and molecular targets. *Cell. Mol. Neurobiol.* **21,** 645–656.

Shir, A., Ogris, M., Wagner, E., and Levitzki, A. (2006). EGF receptor-targeted synthetic double-stranded RNA eliminates glioblastoma, breast cancer, and adenocarcinoma tumors in mice. *PLoS Med.* **3,** e6.

Singh, S. K., Hawkins, C., Clarke, I. D., Squire, J. A., Bayani, J., Hide, T., Henkelman, R. M., Cusimano, M. D., and Dirks, P. B. (2004). Identification of human brain tumour initiating cells. *Nature* **432,** 396–401.

Sordella, R., Bell, D. W., Haber, D. A., and Settleman, J. (2004). Gefitinib-sensitizing EGFR mutations in lung cancer activate anti-apoptotic pathways. *Science* **305,** 1163–1167.

Taketo, M. M. (2006). Mouse models of gastrointestinal tumors. *Cancer Sci.* **97,** 355–361.

Tamura, K., and Fukuoka, M. (2005). Gefitinib in non-small cell lung cancer. *Expert Opin. Pharmacother.* **6,** 985–993.

Tokarski, J. S., Newitt, J. A., Chang, C. Y. J., Cheng, J. D., Wittekind, M., Kiefer, S. E., Kish, K., Lee, F. Y. F., Borzillerri, R., Lombardo, L. J., Xie, D., Zhang, Y., *et al.* (2006). The Structure of dasatinib (BMS-354825) bound to activated ABL kinase domain elucidates its inhibitory activity against imatinib-resistant ABL mutants 10.1158/0008-5472.CAN-05-4187. *Cancer Res.* **66,** 5790–5797.

Tsai, C. M., Levitzki, A., Wu, L. H., Chang, K. T., Cheng, C. C., Gazit, A., and Perng, R. P. (1996). Enhancement of chemosensitivity by tyrphostin AG825 in high-p185(neu) expressing non-small cell lung cancer cells. *Cancer Res.* **56,** 1068–1074.

Vermeulen, K., Berneman, Z. N., and Van Bockstaele, D. R. (2003). Cell cycle and apoptosis. *Cell Prolif.* **36,** 165–175.

Vogelstein, B., and Kinzler, K. W. (2004). Cancer genes and the pathways they control. *Nat. Med.* **10,** 789–799.

von Bubnoff, N., Manley, P. W., Mestan, J., Sanger, J., Peschel, C., and Duyster, J. (2006). Bcr-Abl resistance screening predicts a limited spectrum of point mutations to be associated with clinical resistance to the Abl kinase inhibitor nilotinib (AMN107). *Blood* **108,** 1328–1333.

Voskoglou-Nomikos, T., Pater, J. L., and Seymour, L. (2003). Clinical predictive value of the *in vitro* cell line, human xenograft, and mouse allograft preclinical cancer models. *Clin. Cancer Res.* **9,** 4227–4239.

Warburg, O. (1956). On the origin of cancer cells. *Science* **123,** 309–314.

Warner, J. K., Wang, J. C., Hope, K. J., Jin, L., and Dick, J. E. (2004). Concepts of human leukemic development. *Oncogene* **23,** 7164–7177.

Xia, W., Bacus, S., Hegde, P., Husain, I., Strum, J., Liu, L., Paulazzo, G., Lyass, L., Trusk, P., Hill, J., Harris, J., and Spector, N. L. (2006). A model of acquired autoresistance to a potent ErbB2 tyrosine kinase inhibitor and a therapeutic strategy to prevent its onset in breast cancer. *Proc. Natl. Acad. Sci. USA* **103,** 7795–7800.

Yaish, P., Gazit, A., Gilon, C., and Levitzki, A. (1988). Blocking of EGF-dependent cell proliferation by EGF receptor kinase inhibitors. *Science* **242,** 933–935.

Yeon, C. H., and Pegram, M. D. (2005). Anti-erbB-2 antibody trastuzumab in the treatment of HER2-amplified breast cancer. *Invest. New Drugs* **23,** 391–409.

Yoneda, T., Lyall, R. M., Alsina, M. M., Persons, P. E., Spada, A. P., Levitzki, A., Zilberstein, A., and Mundy, G. R. (1991). The antiproliferative effects of tyrosine kinase inhibitors tyrphostins on a human squamous cell carcinoma *in vitro* and in nude mice. *Cancer Res.* **51,** 4430–4435.

Zhang, X., Gureasko, J., Shen, K., Cole, P. A., and Kuriyan, J. (2006). An allosteric mechanism for activation of the kinase domain of epidermal growth factor receptor. *Cell* **125,** 1137–1149.