

Signal Events: Cell Signal Transduction and Its Inhibition in Cancer

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Key Words. *Signal transduction · Targeted cancer therapy · mTOR · ErbB family · Rapamycin*

LEARNING OBJECTIVES

After completing this course, the reader will be able to:

1. Recognize the importance of identifying new molecular targets for cancer therapy and how they relate to the development of novel therapeutic agents with high therapeutic indices and low toxicity.
2. Explain the process of signal transduction (specifically the ErbB family of receptors and the mTOR pathways) and how it relates to cell growth, differentiation, and survival, and describe the effects of aberrations in signal transduction on the development of neoplasms.
3. Describe some of the novel targeted therapies that have been developed and explain the mechanisms by which signal transduction inhibitors inhibit tumor growth and induce tumor regression in patients with cancer.

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ABSTRACT

Signal transduction refers to communication processes used by regulatory molecules to mediate the essential cell processes of growth, differentiation, and survival. Signal transduction elements interact through complex biochemically related networks. Aberrations in signal transduction elements can lead to increased proliferative potential, sustained angiogenesis, tissue invasion and metastasis, and apoptosis inhibition. Most human neoplasms have aberrant signal transduction elements. Several compounds that target aberrant signal transduction elements, such as those in the ErbB family of tyrosine kinase receptors and mammalian target of rapamycin, are in development. To date, commercially available signal-transduction-targeting compounds include trastuzumab, a monoclonal antibody against the ErbB-2 receptor for the treatment of metastatic breast cancer overexpressing the ErbB-2 (HER-2) receptor, and

gefitinib, an inhibitor of the ErbB-1 receptor tyrosine kinase that recently received regulatory approval for the treatment of patients with non-small cell lung cancer. In contrast to traditional cytotoxic treatments, although signal transduction inhibitors are capable of inducing tumor regression, particularly in malignancies that are principally driven by specific target aberrations, preclinical and early clinical investigations suggest that their predominant beneficial effects are growth inhibitory in nature; therefore, new clinical trial designs and evaluation end points may be required to ultimately assess their value. Prospective profiling of patients and tumors to determine treatment response is also essential to the success of these clinical trials. However, responsiveness to these novel therapies is dependent on a multitude of factors that ultimately determine the robustness and quality of the downstream response. *The Oncologist* 2003;8(suppl 3):5-17

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INTRODUCTION

A plethora of recently acquired information about specific molecular abnormalities that “drive” the malignant phenotype, together with profound advances in biotechnology, has resulted in the beginning of an era of abundant novel therapeutic options to treat patients with a variety of malignant diseases. It has also brought about many unique challenges for clinical investigators. As anticancer therapeutics with distinct targeting capabilities against malignant cells are developed, prioritization of these therapies for efficient allotment of clinical trial resources, identification of patients whose malignancies most likely express the molecular constituents resembling the true target, and derivation of relevant end points for both screening and assessment of clinical relevance will be critical to their successful development and optimization. The predominant biologic effects of these agents in preclinical studies (i.e., tumor growth delay) and their much more disparate effects on malignant and normal tissues compared with nonspecific cytotoxic agents suggest that clinical evaluation and regulatory end points that are generally considered secondary for nonspecific cytotoxic agents, such as improvements in disease-related symptoms and quality of life, may evolve into primary end points similar to the situations typically encountered in the assessment of therapeutic benefit in other medical disciplines.

The cellular processes that are specifically being targeted for therapeutic development are those that are principally responsible for the proliferative advantage inherent in the malignant phenotype, including: aberrant signal transduction (ST); cell cycle dysregulation; evasion of apoptosis; sustained angiogenesis; tissue invasion and metastasis; and immune tolerance [1-4]. The development of novel therapeutic agents that are highly selective between host and tumor should enable the achievement of high therapeutic indices with low toxicity in the treatment of cancer [5].

THE VALIDITY OF RATIONAL THERAPEUTIC TARGETING OF CELL ST SYSTEMS

The term “signal transduction” refers to the means by which regulatory molecules that govern the fundamental processes of cell growth, differentiation, and survival (e.g., extracellular hormones, growth factors, cytokines, and specialized proteins) communicate within the cell, resulting in the tight coordination of proliferative and other essential processes among various tissues. Cellular signaling processes are essential to the life cycle and biologic function of all cells and are critically important in governing processes such as proliferation and differentiation. ST elements interact through biochemical cascades in interrelated networks within cells and among tissues [2, 6]. The robustness and diversity of

these signals are enhanced by many redundant routes within the various pathways and elaborate interconnections among the networks [7].

Most malignancies have aberrant ST elements of variable magnitudes, so they are logical targets for therapeutic development [8]. Highly aberrant ST elements often give rise to constitutive activation of the cell growth process. The autonomy of cell growth is the dominant feature of the malignant phenotype [1, 2, 4]. Aberrant or overexpressed ST elements or growth factor receptors may result in an increased proliferative rate and potential for invasion, metastasis, and angiogenesis—and shift the equilibrium between survival and cell death (apoptosis) control toward survival [1]. Resistance to traditional cytotoxic chemotherapy and radiation therapy may be due, at least in part, to the development of aberrant ST elements; therefore, therapeutics targeting these aberrations may be useful adjuncts to these standard therapies [8].

ST Systems

In contrast to linear systems, evolutionary pressures over millions of years have driven the development of networks of biochemical pathways that provide the critical proliferative signals necessary for growth, survival, and differentiation. As a testament to evolutionary pressures, these signaling pathways are found in network arrays rather than in linear cascades. Thus, the same evolutionary pressure can be held to account for the presence of redundant pathways. These tightly coordinated, efficient, and redundant ST systems allowed for the evolution of tissue-based organisms from single-celled organisms. Ultimately, the inherent redundancy of the network may allow cells to resist toxins and negative evolutionary pressures (Fig. 1) [7]. In most neoplasms, ST appears to be aberrant, with some mutant signaling proteins acting as oncogenes.

Cells are constantly exposed to a variety of external stimuli, ranging from soluble endocrine and paracrine factors to signaling molecules on neighboring cells. Thus, it is extremely important that the cell correctly interprets these extracellular signals to create an appropriate developmental or proliferative response. The most common stimuli use secondary messenger networks that rely upon a relay system for signal amplification and diversity. Examples of relay systems are the seven-transmembrane receptor group [9] and enzyme-linked receptors [6, 10]. These systems process signals by different methods. For the seven-transmembrane receptor group, which includes hormones and neurotransmitters, conformational changes upon ligand binding lead to dissociation of heterotrimeric G-proteins [9]. Enzyme-linked receptors will, upon ligand binding, increase enzymatic activity in the receptor, causing activation. Examples include

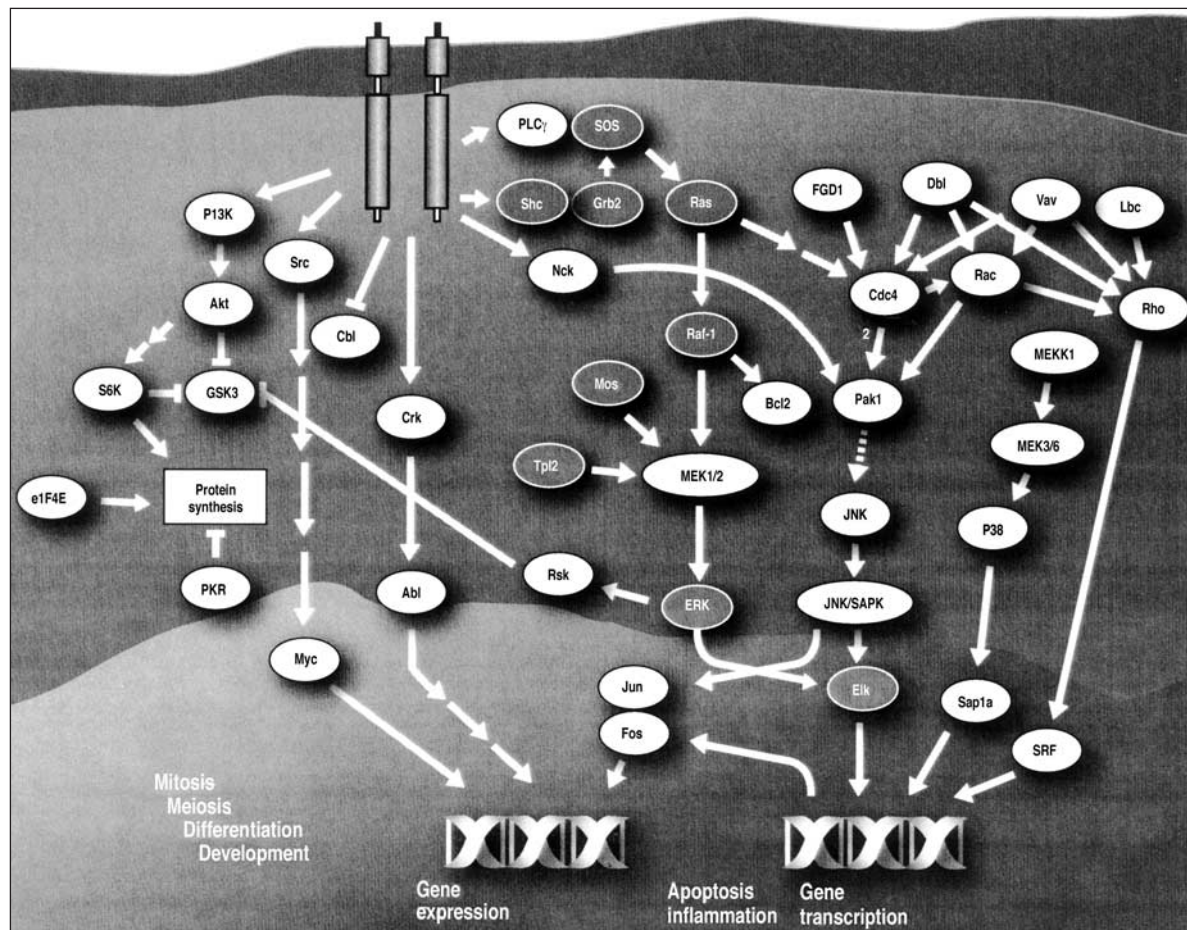


Figure 1. Example of some network cascades related to the mitogen-activated protein kinase and related pathways of ST. Evolutionary pressures have resulted in the conveyance of critical signals by networks, not linear cascades. ST = signal transduction.

systems activated by receptor tyrosine kinases (RTKs), transforming growth factor beta, and cytokines. Receptors of the tyrosine kinase family play principal roles in these processes because they are able to integrate a wide range of external stimuli with specific internal signals and responses, ultimately allowing the cell to respond correctly to its environment.

Other relay systems use methods such as ligand gate ion channels that open or close in response to ligand binding (e.g., postsynaptic receptors) or ST via intermembrane cleavage of the receptor. In the latter case, ligand binding leads to regulated proteolytic cleavage of a transmembrane receptor that releases an intercellular fragment of the receptor into the cytoplasm. The receptor fragment is then translocated to the nucleus, where it regulates a response. Examples of this system include the ErbB family of receptors, CD44, and amyloid precursor protein [7]. Another type of signaling process is by ligand passage through the membrane. This is the process by which thyroid hormones, retinoids, vitamin D receptors, and androgens are transduced. Passage of the

ligand across the membrane induces a conformational change in the cytoplasmic or nuclear receptor, which then becomes activated. In these well-regulated systems, the receptor is often associated with an inhibitor that prevents ligand-independent activation of signaling.

Finally, there is a somewhat controversial proposal that transmembrane receptors may directly signal to the nucleus. This theory was based upon the discovery in the nucleus of transmembrane receptors with and without their ligands. Whether these intranuclear receptors can directly mediate transcriptional responses is unknown.

ST Pathways as Targets for Therapeutics Development

The ultimate end result of ST networks is to regulate cell growth and division. The most common abnormality associated with tumorigenesis is unchecked cell activity and growth, mediated primarily by aberrant and/or overexpressed elements of ST networks. A better understanding of the changes that affect ST pathways has resulted in the evaluation of a variety of new therapeutic targets. Agents that

target these pathways and that are highly specific for various components of these complex biologic processes hold great promise for better patient outcomes. Several of these ST elements and related networks are being evaluated as targets for the development of therapeutic agents that block activation of cell surface receptors. The following discussion focuses on two ST systems: the ErbB receptor family and its downstream elements; and mammalian target of rapamycin (mTOR), which mediates the conduction of signals through several related vital pathways. The complexities of these systems present new challenges to therapeutic targeting and to the clinical development and evaluation of rational, targeted therapies.

ErbB Receptor Family

The ErbB receptor family is characteristic of the RTKs. These receptors are critical for mediating the proliferation and differentiation of normal cells [7, 11-13]. To date, four subfamilies have been described and designated ErbB-1 (also known as human epidermal growth factor receptor [EGFR] or HER-1), ErbB-2 (HER-2), ErbB-3 (HER-3), and ErbB-4 (HER-4). With few exceptions (e.g., hematopoietic cells), the ErbB receptors are expressed in cells of mesodermal and ectodermal origins. In epithelial tissues, the basolateral distribution of ErbB family members enables them to mediate signals required for growth between mesenchymal and epithelial tissue components [14]. The ErbB receptors comprise three structural domains: an extracellular domain for ligand binding, a transmembrane domain, and a cytoplasmic tyrosine kinase domain associated with a tyrosine kinase regulator or receptor. Each subfamily has associated ligands, except for ErbB-2, which may function as a coreceptor for other ErbB subfamilies [15-17].

ErbB receptor function begins upon ligand binding and is followed by receptor dimerization. The dimerization process can occur between two receptors of the same family (heterodimerization, e.g., ErbB-1 and ErbB-3) or between two of the same receptors (homodimerization, e.g., ErbB-1 and ErbB-1) [15]. Stimulation by a specific ligand confers a specific dimerization profile that is tissue specific or tumor specific [15]. The process of dimerization activates the tyrosine kinase domain of the receptor and is followed by the phosphorylation of multiple tyrosine residues, which in turn activates downstream receptor proteins that ultimately lead to physiologic responses [15, 18].

Upon ligand binding, the ErbB receptor becomes activated and downregulated through a series of steps [7, 11-13, 15, 16, 18]. Ligand binding leads to receptor aggregation, which in turn facilitates the formation of both ErbB homodimers and heterodimers. ErbB homodimers and heterodimers are able to activate intrinsic tyrosine kinases of

receptors via intermolecular phosphorylation within their cytoplasmic domains. The resulting phosphorylated tyrosine residues modulate the readiness of, or serve as docking sites for, downstream signaling molecules and cytoplasmic messenger proteins, which then initiate a cascade of signals that emanates from the cytoplasm to the nucleus. Key tyrosine phosphorylation sites responsible for the recruitment of downstream receptor targets are located in the juxtamembrane region and C-terminal tail of the receptor, which flank the tyrosine kinase domain. Signaling through ErbB-1 and other family members triggers a powerful network of downstream cellular pathways, ending in responses that range from cell division to cell death, and from motility to adhesion, and include invasiveness and angiogenesis. Ultimately, effects on gene expression determine the biologic response to receptor activation. Because the network is often dysregulated in human cancers, a molecular comprehension of these processes may lead to the development of new therapeutics with clinical ramifications.

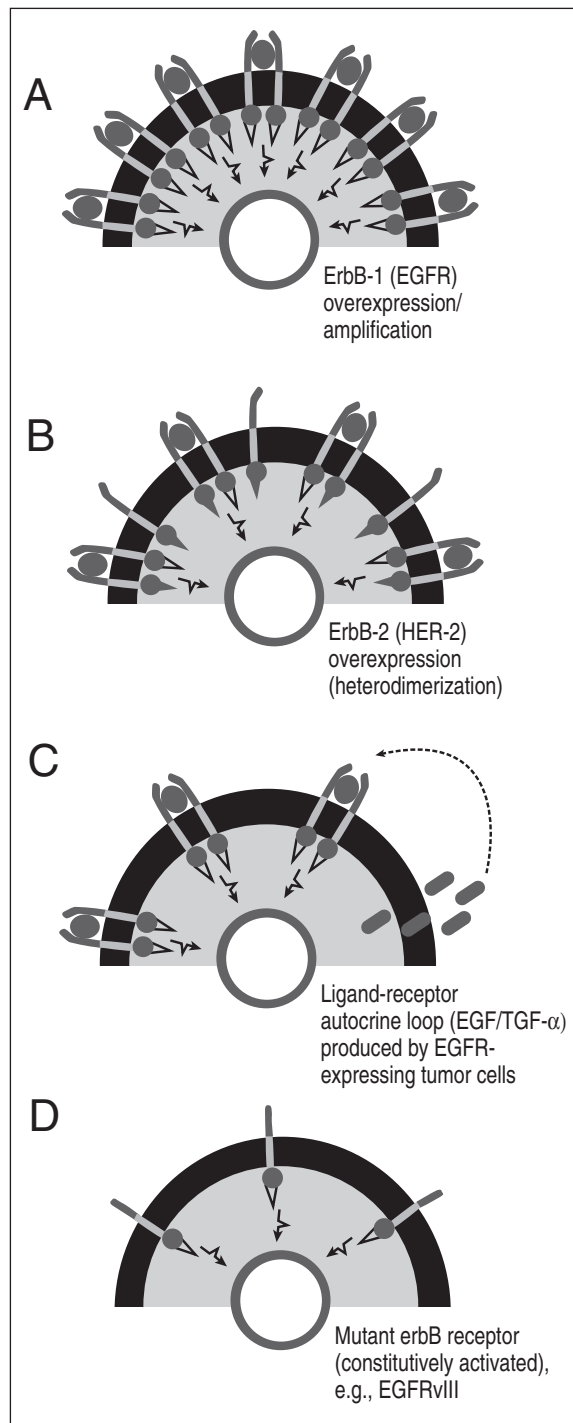
ERBB RECEPTOR SIGNALING MECHANISMS IN HUMAN CANCER

It has been suggested and supported by experimental data that aberrant activation of the kinase activity of ErbB receptors plays a primary role in the development and/or progression of human cancer. The ErbB receptors are associated with a number of tumor types, one example being the well-known HER-2 receptor, which is identified in 20%-30% of invasive breast cancer cases and is associated with a poor prognosis. A variety of different mechanisms leads to increased ErbB receptor activation and, ultimately, increased cellular proliferation (Fig. 2) [10, 18]. Mechanisms associated with the development, as well as the proliferative and survival advantages, of several types of human neoplasms include overexpression of the ErbB receptor protein, the development of ligand/autocrinal feedback loops, and phosphatase deficiencies that lead to decreased receptor degradation. In other cases, tumors may have mutated or truncated receptors that confer constitutive activation of the receptor [19].

Aberrant ErbB subfamilies are associated with the development of a variety of human tumor types, many of which lack effective therapeutic agents [20]. For instance, ErbB subfamilies are overexpressed in breast and colorectal cancers, gastric carcinoma, gliomas, and tumors that arise in mesodermal tissue [15, 16, 18]. In breast cancer, approximately 25% of patients have amplification of HER-2/*neu* genes, leading to overexpression of ErbB-2 [15, 20].

Factors Affecting ErbB Signals

Signals relayed through ErbB receptors can be affected by multiple factors, including receptor expression levels



and constitutive aberrations to the receptor. Several other factors converge to determine the specific quality and magnitude of the signal in the ErbB system. These factors include the nature of the system's ligand, the nature of the dimer upon activation by its ligand, dimer degradation rate, and crosstalk with other pathways (Fig. 3) [7].

The nature of the ligand determines the nature of the dimer formed upon binding; this very complex process

Figure 2. Mechanisms of increased ErbB receptor activation.

A) Overexpression or amplification of ErbB-1 receptor, increasing the potency of responses to available ligands. B) Overexpression of ErbB-2, resulting in preferential formation of ErbB-2 heterodimers with other ErbB receptors or autoactivated ErbB-2 homodimers. C) Ligand-receptor autocrine loop in which both receptor and ligand are produced by the same cell. D) Mutations in receptors, resulting in their constitutive activation, such as occurs with EGFRvIII. EGF = epidermal growth factor; EGFR = EGF receptor; TGF- α = transforming growth factor α . (From Rowinsky EK. Targeting signal transduction: the erbB receptor family as a target for therapeutic development against cancer. In: *Horizons in Cancer Therapeutics: from Bench to Bedside*. Meniscus Education Institute, West Conshohocken, PA. 2001;2:3-36.) Adapted with permission.

involves the possibility of multiple ligands and subsequent dimer profiles that each confer a particular mitogenic response to the cell [7]. Therefore, the magnitude and type of mitogenic response downstream is a contextual summation of the individual effects of multiple ligands and receptor dimerization profiles. Consequently, targeting one specific ErbB subfamily may be insufficient to impart a significant therapeutic benefit [15, 21].

The magnitude of signals transduced differs among dimer profiles, for example, between homodimers and heterodimers. The homodimer ErbB-3 does not confer any signaling, while both ErbB-1 and ErbB-4 are weak signaling homodimers [20]. Conversely, ErbB-2 is a preferred heterodimer partner that conveys a potent signal [20]. The specific features of ErbB-2 heterodimers that allow for potent signaling include slow ligand dissociation, relaxed ligand specificity, rapid recycling of the ligand, and prolonged firing of the ligand [10, 20]. Taken together, the ErbB-2

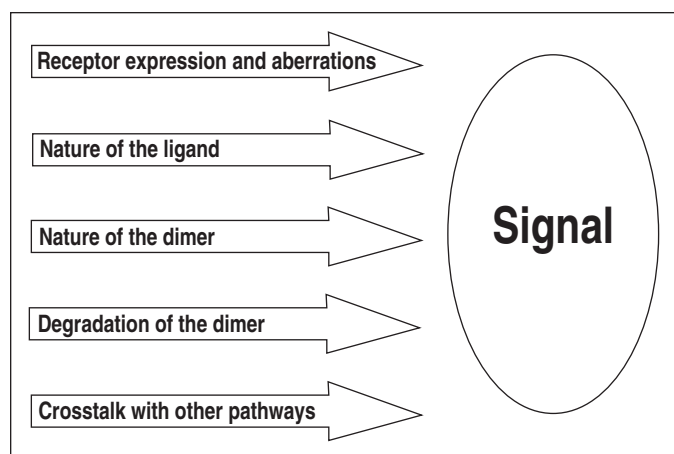


Figure 3. Factors determining the quality and magnitude of the ErbB signal. The magnitude and quality of the signal reflect a contextual summation of many determinants.

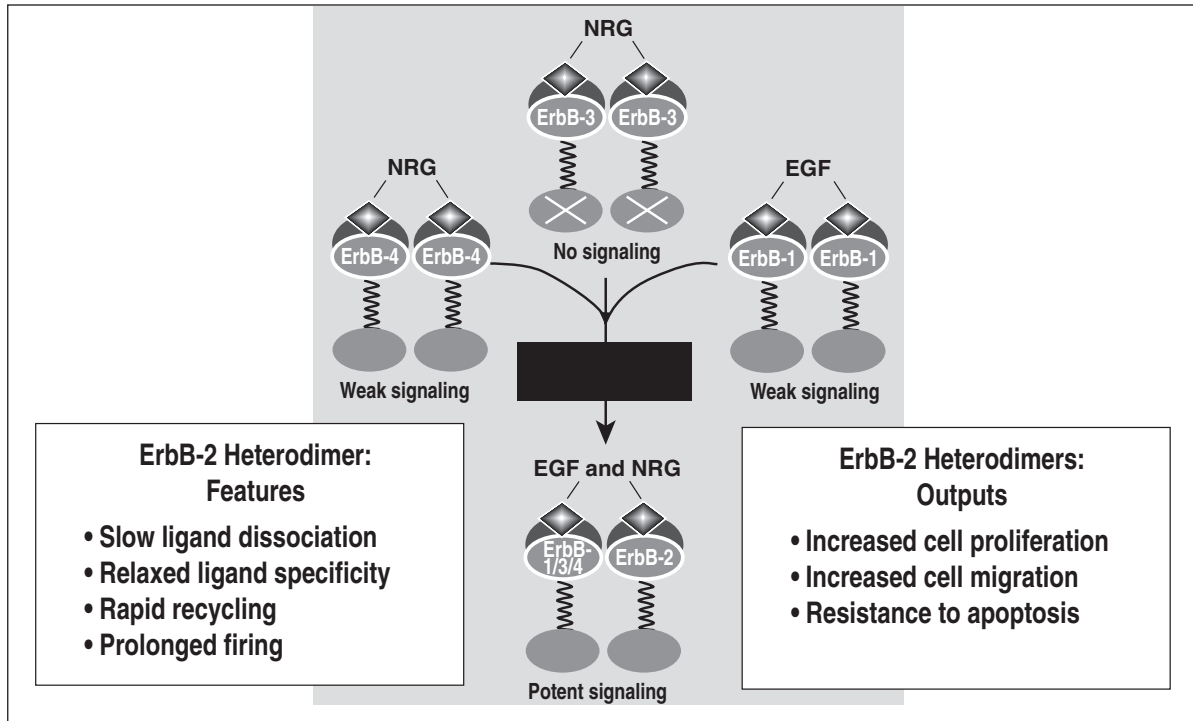


Figure 4. Signaling as a function of the nature of the dimer. ErbB-2 heterodimers amplify signaling and result in a more potent response than homodimers. EGF = epidermal growth factor; NRG = neuregulin. (From Yarden Y, Sliwkowski MX. *Untangling the erbB signaling network. Molecular Cell Biol* 2001;2:127-137. Adapted with permission.)

heterodimer features lead to dramatic increases in cell proliferation and cell migration, as well as resistance to apoptosis [7, 10, 16] (Fig. 4).

In addition to the dimer profile, the conformation of the phosphotyrosine groups on the activated ErbB receptor determines which downstream adaptor protein will react with it. For example, the ErbB-1-ErbB-3 heterodimer preferentially provides docking sites for the phosphoinositide-3 kinase (PI3K) adaptor proteins, while the ErbB-2-ErbB-1 heterodimer leads to preferential activation of the PI3K pathway, resulting in the phosphorylation of a variety of survival proteins and increased translation of cell cycle proteins [10].

Another determinant of the quality and magnitude of the downstream signal is the rate of dimer degradation, which is an endocytotic process [10, 22]. Dimer degradation is initiated by ligand binding, which then induces receptors to cluster in clathrin-coated membrane pits. This process is followed by endocytosis and eventual lysosomal degradation of the dimer. The rate of endocytosis determines the amplification or attenuation of the signal [6, 7]. Dimer degradation is also dependent on the nature of the dimer and its tyrosine kinase activity. For example, kinase-negative mutants recycle to the cell surface for reutilization and are, therefore, not preferentially degraded [11].

The receptor degradation process effectively “turns off” the ErbB response; consequently, it is a valuable target for rational therapy. One such therapeutic target is the signaling protein Cbl, which is important in receptor processing. The Cbl protein attracts ubiquitin-loaded molecules that tag the receptor with ubiquitin for recognition and sorting, eventually leading to proteosomal digestion [6]. Currently, rational therapeutics to target dimer degradation are in development. For example, the irreversible, pan-ErbB tyrosine kinase inhibitor CI-1033 (Pfizer Inc.; Groton, CT) is a strong inducer of poly-ubiquitylation and ErbB-2 degradation [23].

Finally, the integration of heterologous signals outside the ErbB signaling system, that is, crosstalk with pathways outside its system, also can affect the quality and magnitude of ErbB signals [7]. For example, lysophosphatidic acid, endothelin, and thrombin can activate G-protein-coupled receptors (GPCRs) that, in turn, untether membrane-bound ErbB ligands, such as heparin-bound epidermal growth factor (HB-EGF), subsequently freeing them to bind to ErbBs. Activation of GPCRs may then activate Src kinases, leading to phosphorylation of tyrosine residues on the intracellular ErbB domains. These actions set in motion downstream ErbB-1 events that may contribute to the mitogenic potential of heterologous agonists [24].

Table 1. Antibodies to ErbB receptors in clinical development			
Agent	Class	Phase of development	Molecular target
Trastuzumab	Humanized recombinant IgG _{1κ} antibody (chimera)	Regulatory approval: breast Phase III: NSCLC	Binds to external domain of ErbB-2
Cetuximab	Chimeric antibody	Preregistration: colorectal Phase III: head and neck, NSCLC Phase II: breast, pancreatic, prostate	Binds to external domain of ErbB-1
EMD 72000	Humanized recombinant antibody	Phase II: gastrointestinal, cervical, NSCLC Phase I: head and neck, ovarian, pancreatic	Binds to external domain of ErbB-1
h-R3	Humanized recombinant antibody	Phase II: head and neck	Binds to external domain of ErbB-1
ABX-EGF	Human IgG ₂ antibody	Phase II: renal, NSCLC, colorectal, prostate Phase I: pancreatic, esophageal	Binds to external domain of ErbB-1
MDX-447	Bispecific monoclonal antibody	Phase II: head and neck, renal, breast, bladder, ovarian	Binds to both ErbB-1 and CD64 on neutrophils and monocytes

ErbB-Targeted Therapeutics in Development

Several therapies have been developed that specifically target the ErbB receptor family. A variety of strategies have been considered for inhibiting ErbB receptor activity. In one strategy, an antibody binds to the receptor, blocking ligand binding and/or accelerating receptor internalization and degradation. Another strategy involves the utilization of a low-molecular-weight inhibitor of RTK activity, which blocks receptor activation. In a third strategy, a bispecific antibody binding to a receptor and an immune cell facilitates immunologic attack. In a fourth approach, an anti-receptor antibody conjugated to molecules of a cellular toxin or cytotoxic drug promotes receptor internalization and delivery of the drug or toxin to the interior of the cell. Finally, antisense oligonucleotides complementary to the nucleotide sequence of the ligand or receptor block protein translation.

The first ErbB receptor inhibitor to be approved by the U.S. Food and Drug Administration (FDA) was the monoclonal antibody trastuzumab (Herceptin[®]; Genentech, Inc.; South San Francisco, CA). This agent is indicated for the treatment of patients with metastatic breast cancer whose tumors overexpress the ErbB-2 (HER-2/*neu*) receptor [7, 25, 26]. Currently, many other ErbB receptor-targeted therapies are in development, including monoclonal antibodies and small molecules that target the tyrosine kinase domain of the receptors [10, 17, 27-30]. Other compounds are being developed to target only the external domain of ErbB-1, such as cetuximab (Erbix[®], IMC C-225; ImClone Systems, Inc.; New York, NY), EMD 72000 (Merck Kga; Darmstadt,

Germany), ABX-EGF (Abgenix, Inc.; Fremont, CA), and MDX-447 (Medarex Inc.; Princeton, NJ). Another approach is to target the tyrosine kinase domain of ErbB-1, as exhibited by gefitinib (Iressa[®]; AstraZeneca Pharmaceuticals; Wilmington, DE), which recently received regulatory approval in the U.S. for the treatment of patients with non-small cell lung cancer (NSCLC) resistant to platinum agents and docetaxel, and by erlotinib (Tarceva[™]; OSI Pharmaceuticals; Melville, NY) [27, 31]. Yet other agents target multiple ErbB receptor subfamilies. The compound GW572016 (GlaxoSmithKline; Middlesex, UK) is an example, as it targets the RTKs of both ErbB-1 and ErbB-2 [10]. CI-1033 and EKB-569 (Wyeth-Ayerst; Philadelphia, PA) bind irreversibly to the ATP-binding site and inhibit the tyrosine kinase activity of multiple ErbB subfamily members [32, 33]. Table 1 summarizes some of the antibodies to ErbB receptors in clinical development.

Therapeutic Development Considerations

As described above, several factors are being considered in the development of targeted therapeutic agents for cancer. For example, some compounds in development target multiple ErbB family receptors in an attempt to circumvent the redundancies inherent to this system. Other compounds (e.g., CI-1033 and EKB-569) create an irreversible bond by covalently binding to the receptor, thereby facilitating continuous tyrosine kinase inhibition in the face of very high intracellular concentrations of ATP [34]. However, the relative merits of irreversible binding to ErbB are unknown and may depend on the half-life of the agent. The relatively long

half-lives of nonalkylating or reversible tyrosine kinase inhibitors may result in greater benefits than those of irreversible compounds with shorter half-lives. Additionally, the average receptor turnover time of 24-36 hours may also negate the benefits of irreversible compounds.

The magnitude and quality of the downstream response to targeted therapies may be determined by a variety of factors that need to be identified in tumors. Thus, another important consideration in the development of targeted therapeutic agents is tumor markers; that is, the subcellular determinants of the ErbB inhibitory response, such as phosphorylated (p)-ErbB-1, p-Erk, and p-Akt. Following treatment with a particular compound, immunohistochemical staining of receptors, phosphorylated aspects of receptors, and ST elements has permitted semiquantitation of these determinants [35].

Clearly, there are many different approaches to developing targeted therapies, and the relative merits of each of these approaches have yet to be determined. Prospective profiling of tumor types in large studies would help researchers to understand the complexities of the systems involved and to determine the links between tumor profiles and responses to treatment. However, this is a considerable enterprise that has yet to be undertaken extensively.

Tumor Growth Inhibition and Regression

While the predominant favorable effects of the ErbB inhibitors are tumor growth inhibitory in nature, overt tumor regression has occurred with many of these novel treatments, even in some well-developed tumors. For example, ABX-EGF and gefitinib have been shown to induce regression in A431 cancer xenografts, which overexpress ErbB-1 (EGFR) and its ligand [10]. It is likely that tumors that are principally driven by aberrations or overexpression of elements of the ErbB ST pathway will demonstrate the most vivid responses (i.e., regression, profound tumor growth delay) in preclinical and clinical studies. Additionally, encouraging response rates have been reported preliminarily following the treatment of several types of tumors, such as advanced and previously treated NSCLC and ovarian, head and neck, and brain (glioblastoma multiforme) carcinomas, with ErbB-targeted therapies [36-38]. Although each of these tumors has been shown to display EGFR RNA and/or protein overexpression [36], it is not known whether these tumors are driven by this phenotype, and the reported efficacy could not have been predicted based on preclinical data. Thus, ErbB-targeted therapies offer clinicians new therapeutic approaches to the treatment of tumors that can lead to tumor regression, tumor growth delay, and symptomatic improvement in some patients. However, the determinants of clinical benefit are not known, which emphasizes the need to prospectively characterize tumors.

MOLECULAR TARGET OF RAPAMYCIN

Another area of research is the development of inhibitors of mTOR, a downstream ST element in the PI3K pathway and a member of the recently identified family of protein kinases termed PI3K-related kinases. These kinases are involved in a number of critical regulatory cellular functions concerning cell cycle progression and cell cycle checkpoints that govern cellular responses to DNA damage, repair, and recombination [39]. mTOR regulates essential ST pathways and is involved in the coupling of growth stimuli with cell cycle progression. PI3K/protein kinase B (Akt) appears to be the key modulatory factor in the upstream pathway by which growth factor-growth factor receptor interactions affect the phosphorylation state of mTOR [40, 41]. PI3K plays a central role in cellular proliferation, cell adhesion, catabolism, and apoptosis, and is upregulated in cancer cells [42]. Activation of the PI3K pathway leads to the production of secondary messengers downstream that activate proliferative elements, for example, Akt and p70^{s6k} [8, 27, 43]. In turn, these elements initiate a variety of local responses, including polymerization of actin, assembly of signaling complexes, and priming of protein kinase cascades [43]. In particular, phosphorylation of Akt stimulates its catalytic activity, leading to the phosphorylation of a number of other proteins that affect cell growth, cell cycle entry, and cell survival. Thus, inhibition of mTOR could eliminate the transduction of proliferative signals and thereby inhibit tumor growth where aberrant signals or mutations occur.

mTOR lies at a critical branching junction in the pathway between the PI3K/Akt/PTEN signaling pathway and downstream proliferative elements [27], and serves to regulate growth stimuli and subsequent cell cycle progression. Experimental data demonstrate that mTOR functions downstream of the PI3K/Akt pathway and is phosphorylated in response to stimuli that activate the PI3K/Akt pathway [40, 41, 44]. Upon stimulation, mTOR phosphorylates a variety of downstream proteins that augment or activate the translation of proteins that are important in the G₁-to-S phase traverse and ribosome biogenesis [8, 45]. There are other signaling pathways that are activated downstream of PI3K, but the Akt pathway is of primary interest because of its role in inhibiting apoptosis and promoting cell proliferation by affecting the phosphorylation status of cell-survival and apoptosis-induction proteins such as BAD [46]. Upstream abnormalities can also activate mTOR and lead to proliferation.

Rapamycin, an mTOR Inhibitor

Rapamycin (Rapamune[®], sirolimus; Wyeth Laboratories; Philadelphia, PA) is a macrocyclic lactone found in the *Streptomyces hygroscopicus* organism. Originally identified

as an antifungal agent, it was subsequently found to have potent immunosuppressant properties, leading to its approval for the prophylaxis of organ rejection in patients receiving renal transplants. More recently, rapamycin has been found to have potent and broad antineoplastic activity as well, and is being developed as a target for mTOR [8, 47]. Rapamycin forms a functional inhibitory unit by binding with receptor FKB-12. This unit subsequently binds with and inhibits mTOR [8]. Together, these actions effectively inhibit phosphorylation of downstream proteins that ultimately block the translation of the G_1 critical proteins necessary for the G_1 -to-S phase traverse and ribosome biosynthesis [8].

Some cell abnormalities and mutations have a hypersensitivity to rapamycin and its analogs [48]. In particular, aberrations in the *PTEN* tumor suppressor oncogene, prostate cancer xenograft, and hyperactivated Akt are hypersensitive to the rapamycin analog CCI-779 (Wyeth-Ayerst; Collegeville, PA), which is currently in broad clinical evaluations [8]. The *PTEN* tumor suppressor gene can be inactivated by deletions, mutations, and hypermethylation. Aberrations in *PTEN* are found in a wide variety of sporadic and inherited neoplasms [48]. Solid tumors with high frequencies of *PTEN* mutations or deletions include glioblastomas (27%-44%), prostate tumors (43%-50%), and endometrial tumors (up to 50%). However, the frequency of *PTEN* mutations is comparatively lower in endometrial, breast, bladder, and lung cancers, and in melanomas and lymphomas [48-50].

mTOR Inhibitors in Development

The unfavorable pharmaceutical properties of rapamycin (poor aqueous solubility and instability) precluded its clinical development as an antineoplastic agent [8]. Thus, soluble ester analogs were synthesized and evaluated in an effort to overcome these pharmaceutical barriers. Rapamycin esters with improved i.v. administration, such as CCI-779 and RAD001 (Novartis Pharmaceuticals Corporation; East Hanover, NJ), and AR23573 (Ariad Pharmaceuticals; Cambridge, MA) are currently in clinical development. Studies of CCI-779 in vitro and in vivo have demonstrated that a number of human and mouse tumors are sensitive to this compound [8, 48].

In tissue culture studies, several cancer cell lines, including human prostate, breast, small cell lung carcinoma, glioblastoma, melanoma, and T-cell leukemia, have shown a high sensitivity to CCI-779. Human tumor xenografts treated with CCI-779 also showed significant growth inhibition, although the predominance of tumor growth inhibition, rather than overt tumor regression, advocates that subsequent disease-directed trials should be specifically designed to detect this potential outcome (i.e., randomized trials with well-designed control arms) [8].

Further, several intermittent CCI-779 dosing regimens were shown to be effective in human tumor xenograft studies, which is important in that extended immunosuppression may result from both rapamycin and CCI-779 administered using a continuous-dose schedule and because the immunosuppressive effects of rapamycin analogs have been shown to resolve within 24 hours following treatment [51].

CCI-779 has been evaluated in two phase I studies, in which the agent was administered as a 30-minute i.v. infusion weekly and as a 30-minute i.v. infusion daily for 5 days every 2 weeks [52, 53]. Those studies were designed to determine the maximum-tolerated dose based on classically defined dose-limiting toxicities. In those studies, the primary toxicities included dermatologic toxicity, myelosuppression, reversible increases in liver function tests, and asymptomatic hypocalcemia. However, the majority of these toxicities were mild to moderate in severity and the maximum-tolerated dose had not yet been determined for CCI-779 administered weekly. In addition, tumor regression of a variety of tumors was observed, including partial responses in patients with previously treated renal cell carcinoma and non-small cell lung carcinoma, and minor responses in previously treated patients with soft tissue sarcoma, serous papillary carcinoma of the endometrium, breast carcinoma, squamous cell carcinoma of the skin, and non-Hodgkin's lymphoma [8]. In early phase II studies, tumor regression has been consistently observed in patients with breast and renal carcinoma, and a phase III study is under way in patients with renal cell carcinoma [54]. The fact that CCI-779 produced tumor regression at relatively nontoxic doses in these trials suggests that the optimal therapeutic dose of this agent may be lower than the maximum-tolerated dose [53].

CLINICAL TRIAL DESIGN

Numerous challenges are evident in the clinical development process of ST inhibitors, all of which may make the clinical trial process more difficult both to plan and to execute. These challenges include defining the optimal doses and administration schedules associated with maximal antitumor activities and minimal toxicities, determining long-term toxicity, and incorporating optimal and sound end points into clinical evaluations based on expectations determined in preclinical studies. Finding solutions to these obstacles may affect the time required to make appropriate go/no go decisions, and to obtain regulatory approval for these agents.

Phase I and Feasibility Studies

The toxicity of traditional, nonspecific cytotoxic agents in rapidly growing tissues is loosely related to antineoplastic activity, and so it can be used as an approximate measure

of drug effect. In contrast, selecting an optimal dosage for ST inhibitors in disease-directed studies is much more difficult. Toxic effects may not appear at doses that effectively inhibit ST or may not even be related to target inhibition. While the results of pharmacologic studies may be used to evaluate the comparative activities of the therapeutic agent in patients versus animals, interspecies differences in tissue drug distribution, protein binding, clearance, and metabolism may preclude the direct extrapolation of data from animals to humans, thereby limiting the usefulness of pharmacologic comparisons. The development and validation of assays reflecting relevant drug effects in accessible tissues should smooth the progress of efforts to define optimal dosing regimens of ST inhibitors in phase I trials. Following the appropriate validation to reflect the desired target effect, such assays may aid the selection of dosages with the highest likelihood of achieving maximal target inhibition.

In addition to dosage, determining optimal administration routes and schedules for ST inhibitors is of primary importance. Current data suggest that continuous long-term treatment may be highly effective in achieving maximal and sustained efficacy. However, extended treatment durations may lead to acquired drug resistance, as well as potentially exposing patients to unique toxic effects. Both of these concerns must be addressed when defining optimal dosing schedules for these agents. Notably, any long-term toxicities associated with continuous long-term treatment with ST inhibitors may not be identified using standard preclinical toxicology studies, which primarily focus on highly proliferative tissues. For ST inhibitors, as well as any other rationally designed, target-based therapeutic agents, organs in which the target is highly expressed or tissues that play a role in the function(s) of those organs will require careful monitoring.

Disease-Directed Screening Evaluations

In addition to determining safety and pharmacokinetic profiles, one of the goals of phase II screening evaluations is to accurately gauge the potential for a given therapeutic agent to produce relevant clinical efficacy. Historically, the key end point for these studies was objective tumor regression, defined using standard criteria of clinical effect that have been generally validated for nonspecific antineoplastic agents. However, tumor regression does not equate to efficacy or clinical benefit, which can be conferred by achieving end points that reflect tumor growth delay (i.e., increased time to tumor progression and survival, as well as improvements or delay in symptoms) and sustained quality of life. Despite this apparent incongruity, tumor regression has proven useful as an end point in phase I/II studies to screen nonspecific cytotoxic agents because of its rough correlation with overall clinical benefit and utility. Thus,

although resource-intensive randomized trials are the only unequivocal method to demonstrate the activity of an agent in terms of time to progression and survival, tumor regression has been widely used as an end point in nonrandomized studies to screen nonspecific cytotoxic agents for potential clinical activity. Of note, imatinib, an RTK inhibitor, was approved by the FDA for the treatment of gastrointestinal stromal tumors (GISTs) based on the results of a randomized trial in which tumor regression was the primary end point [55,56]. However, complete follow-up and submission of mature data on response rate, response duration, and survival, as well as submission of data from subsequent phase III randomized trials were required by the FDA as a condition of the approval [56].

Clinical End Points

Challenges involved in designing disease-directed studies of ST inhibitors correlate primarily with the difficulty of defining appropriate end points to evaluate the relative merits of agents that often produce limited or no tumor regression. While many ST inhibitors have been shown to induce regression of experimental tumors [31, 57-59], the predominant effect in preclinical studies is tumor growth delay, which still may produce clinical efficacy in terms of greater time to progression and survival, particularly if the agent portends minimal toxicity.

Delayed tumor growth can exhibit in at least three discrete circumstances. In the first, treatment does not completely stop tumor growth but decreases growth rate. In this setting, the degree of antiproliferative effect may not be evident to the clinician who cannot objectively measure drug-induced effects on the rate of tumor growth when obvious regression has not been demonstrated. Instead, the clinician may interpret *any* tumor growth as disease progression or treatment failure, although the decrease in tumor growth rate may result in increased time to tumor progression or overall survival, in addition to a global improvement in quality of life for the patient. In the second circumstance, a more significant antiproliferative effect occurs when the rates of tumor cell proliferation and cell death are equivalent, often interpreted as stable disease. In this setting, the clinician is likely to continue treatment as long as the patient does not demonstrate intolerable adverse effects. Although the results of preclinical studies suggest that these first two circumstances are likely to be the most common following treatment with ST inhibitors, the beneficial effects may not be obvious or unequivocally attributed to the agent in nonrandomized phase II screening studies. In the third, less common circumstance, the ST inhibitor significantly inhibits tumor cell proliferation and/or enhances tumor cell death, resulting in net tumor regression. This is most likely to occur when the target is a major driver of tumor proliferation.

Thus, designing phase II and III disease-directed studies to assess the relevant antitumor activities of ST inhibitors is a daunting task. Although many of these agents may be able to induce tumor regression in animals, tumor growth inhibition may not be the principal therapeutic effect in human cancers where the tumor is not driven by a single primary anomaly but by multiple causative anomalies of the specific target. Therefore, clinical situations that are sufficiently sensitive to detect a relevant magnitude of tumor growth inhibition will need to be incorporated into disease-directed clinical evaluations. Understanding the biology of the target is paramount with regard to selecting tumors that are most apt to be driven by the target in early screening studies.

Tumor growth delay as the primary benefit of ST inhibitors offers a new challenge for the selection of appropriate end points for phase II and III studies, specifically because only randomized clinical trials can unequivocally demonstrate such effects on tumor growth. In reality, however, some type of “lead” or indication that the ST inhibitor possesses relevant clinical activity, with the ability to modify the natural history of disease progression, ultimately will need to be observed before resource-intensive, large, randomized, phase III trials are initiated. One way of obtaining such a lead is to compare the relative time to tumor progression in patients receiving single-agent treatment with an ST inhibitor against that resulting from treatment with a relevant standard therapy or supportive care, measured just prior to administration of the experimental agent [60]. Using experience with agents that were later shown to have relevant clinical activity in randomized trials, a 30% prolongation in the time to progression may be a reasonable threshold to use before proceeding to phase III trials. As an alternative, “exploratory” single-arm or randomized phase II trials designed with sufficient power to detect and quantify tumor growth inhibition may provide meaningful leads regarding activity prior to phase III randomized studies. For example, in advanced pancreatic cancer, the percentage of patients surviving at least 1 year in exploratory nonrandomized studies may be considered a reasonable end point to gauge whether to proceed with randomized phase III trials.

The randomized discontinuation trial has been proposed as a potentially highly efficient method to detect drug effects on time to progression, survival, and symptoms. In this design, all patients receive the study drug but only patients who do not demonstrate tumor progression are randomized to treatment with or without the study drug. On a similar note, the proportion of patients with progressive disease as their best response appears to inversely correlate with the ultimate utility of any specific agent in a given clinical setting, and a maximum acceptable threshold of patients with progressive disease as their best response may be a valuable predictor of

the potential usefulness of the agent [61]. Such benchmarks, once validated, may be effective in screening ST inhibitors prior to initiating large, randomized, phase III trials. Finally, for agents that are capable of inducing a low level of tumor regression in preclinical evaluations, large phase II studies may be necessary to detect this low level of activity with sufficient confidence intervals.

Additional surrogate end points that may be considered for efficacy in phase II trials include assessment of target inhibition, relevant changes on positron emission scanning (PET) that reflect decreased cell proliferation, and decrements in tumor markers. While all these potential end points remain intriguing for future trials, only changes on PET scanning have been associated with tumor regression (or progression) in a randomized clinical trial evaluating the efficacy of imatinib for the treatment of GISTs [55], and none of these surrogate end points have been validated in a wide range of tumor types or in a large population of patients. Thus, the challenge is to successfully integrate these proposed end points as new paradigms for evaluating these novel agents.

The primary end points for phase III trials will continue to be based on those reflecting survival. However, the relatively low toxicity of ST inhibitors may allow for more emphasis on other end points related to clinical benefit, such as time to progression, performance status, disease-related symptoms, and quality of life. Further, preclinical data and early clinical results indicate that major tumor regression is unlikely to be the primary effect of ST inhibitors. Because clinical trials are often conducted in patients with advanced disease who require cytoreduction for clinical benefit, reasonable developmental strategies will likely involve evaluations of ST-targeted agents in combination with other therapeutic modalities, particularly because a number of these therapeutic agents have shown synergistic, additive, and supra-additive activities when combined with radiation and a variety of chemotherapeutic agents.

SUMMARY

Within only a few years, anticancer therapeutic development has moved from almost a standstill, with a paucity of new agents showing potential for major effect, to the rapid development of agents targeted against the inherent basis of cancer. This transition is based largely on the exponential rate of information acquisition regarding the cancer cell, particularly in terms of aberrant growth ST and the microenvironment of the cell. Because the ultimate goal of any signaling pathway is to regulate cell growth and division, much of the investigation into novel anticancer agents has focused on the development of ST inhibitors. Examples of some key signaling pathway elements currently being targeted with specific therapeutics include the ErbB receptor family and mTOR. Therapeutic agents targeting these signaling pathways should

provide greater specificity, less toxicity, and higher therapeutic indices. However, adequately designed clinical trials are necessary to ensure that the usefulness of ST inhibitors is correctly evaluated and that potentially useful agents are not rejected solely on the basis of poor performance in an

inadequately designed trial with an inappropriate clinical or biologic end point. The full potential of these new agents may only be realized with the implementation of radically different therapeutic development, evaluation, and treatment paradigms.

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