

## SECTION 9

Patricia M. Lorusso, Anderson J. Ryan, Scott A. Boerner, and Roy S. Herbst

## Small-Molecule Tyrosine Kinase Inhibitors

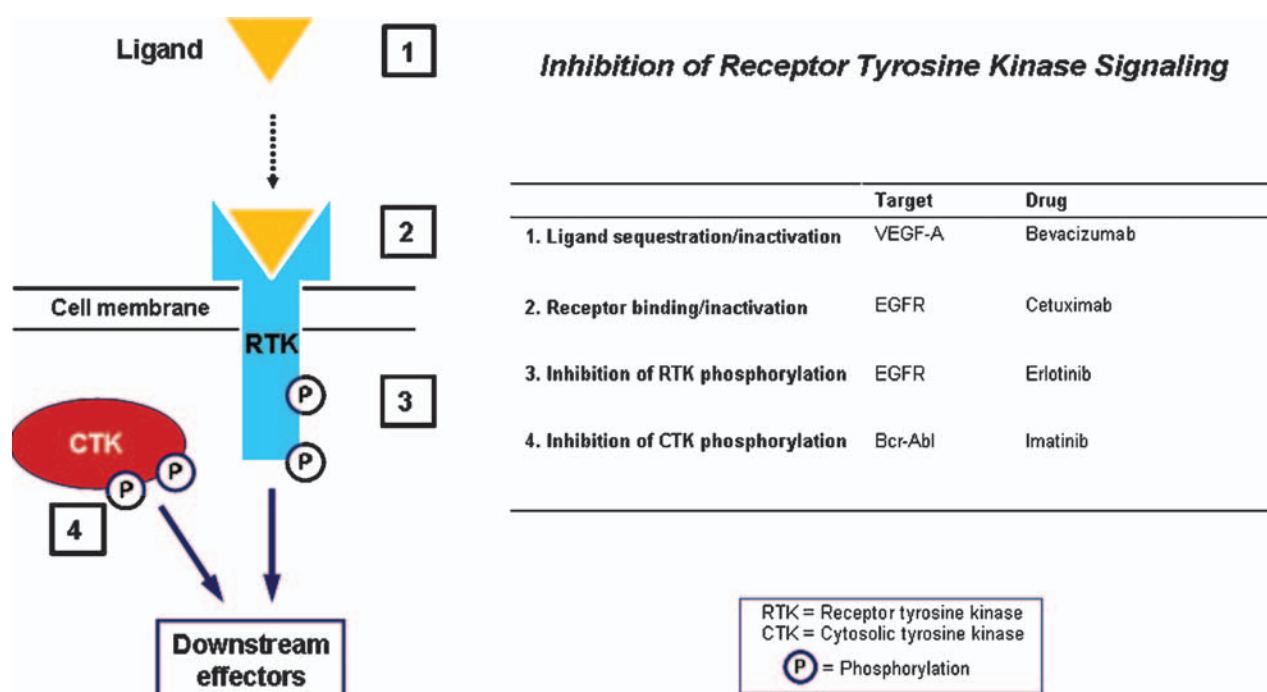
The unveiling of the human cancer genome has had a significant impact on cancer drug development. The identification of genes and pathways involved in oncogenesis has led to a more target-driven drug developmental process, replacing the cytotoxic drug screening approach. Treatments with traditional chemotherapies often result in deleterious side effects caused by their lack of discrimination between cancer cells and normal, rapidly dividing cells in the body. Therapies that selectively target tumors offer the promise of limiting “collateral damage” to normal cells in the body, reducing side effects while increasing the therapeutic window. Many pathways involved in oncogenesis are driven by tyrosine kinases (TKs). TKs, a subclass of protein kinases, are a group of enzymes that catalyze the phosphorylation of tyrosine residues in a protein and govern a multitude of basic cellular activities, including growth, survival, proliferation, differentiation, and apoptosis. Of the approximately 518 kinases found to be encoded in the human genome, 90 TKs have been identified.<sup>1</sup>

In recent years, TKs have increasingly become an important focus in targeted drug development.<sup>2-6</sup> There are two main groups of TKs: receptor TKs and nonreceptor (cellular) TKs. Receptor TKs, which consist of an extracellular domain, a transmembrane domain, and an intracellular domain, are stimulated by growth factors, and recruit a series of downstream effector molecules to conduct complex activation pathways. Nonreceptor TKs are present within the cytoplasm, nucleus, or the intracellu-

lar portion of the plasma membrane. Several TKs have been implicated in oncogenesis, and research in aspects of cell signaling has allowed insight into how aberrant activation of signaling cascades originating from TKs contributes to the formation of tumors. Thus, antitumor properties resulting from TK inhibition is an important focus for drug development. Two methods of inhibiting TK activation have been used (Fig. 25.9.1).<sup>2</sup> First, monoclonal antibodies have been used to compete for the extracellular ligand domain of receptor TKs through ligand sequestration (e.g., bevacizumab) or receptor binding (e.g., cetuximab). These antibodies limit binding of the actual ligand and prohibit activation of the ensuing signal cascade.

Several monoclonal antibodies directed against the extracellular domain of receptor TKs have shown promise against a variety of tumor types; however, most kinase activities are located in the intracellular domain. The second method of blocking TK activation is through the use of agents that prohibit the phosphorylation of intracellular tyrosine residues located on receptor TKs (e.g., erlotinib) or cytosolic TKs (e.g., imatinib) through the blocking of their adenosine 5'-triphosphate (ATP)-binding sites (Fig. 25.9.1). These agents are referred to as *small-molecule tyrosine kinase inhibitors* (TKIs).

TKs and other protein kinases were initially deemed to be poor targets for drug development because of their identical catalytic mechanisms, high degree of sequence homology, similar



**Figure 25.9.1.** Inhibition of receptor tyrosine kinase signaling. VEGF, vascular endothelial growth factor; EGFR, epidermal growth factor receptor.

**TABLE 25.9.1** Food and Drug Administration (FDA)-Approved Tyrosine Kinase Inhibitors (TKIs)

TKI	Known Target(s)	FDA-Approved Indication(s)	Dose/Schedule
Imatinib mesylate	Bcr-Abl, PDGFR, and c-kit	<ol style="list-style-type: none"> <li>1. First-line treatment of Ph-positive CML in blast crisis, accelerated phase, or chronic phase after failure of interferon-<math>\alpha</math> therapy.</li> <li>2. First-line treatment of kit (CD117)-positive unresectable and/or metastatic malignant GIST.</li> <li>3. Ph-positive CML in chronic phase in pediatric patients after recurrence after stem cell transplant or resistance to interferon-<math>\alpha</math> therapy.</li> </ol>	Adult CML: 400 mg/day for chronic phase, 600 mg/day for accelerated phase or blast crisis GIST: 400 or 600 mg/day Pediatric CML: 260 mg/m <sup>2</sup> /day given once per day or split into two daily doses
Dasatinib	Bcr-Abl, Src family, c-kit, EphA2, and PDGFR $\beta$	<ol style="list-style-type: none"> <li>1. Adults with all phases of CML with resistance or intolerance to prior therapy, including imatinib.</li> <li>2. Adults with Ph-positive ALL with resistance or intolerance to prior therapy.</li> </ol>	70 mg twice daily (140 mg/day)
Gefitinib	EGFR	Adults with locally advanced or metastatic NSCLC who have failed platinum-based and docetaxel chemotherapies	250 mg/day
Erlotinib	EGFR	<ol style="list-style-type: none"> <li>1. Patients with locally advanced or metastatic NSCLC after failure of at least one prior chemotherapy regimen.</li> <li>2. In combination with gemcitabine for the first-line treatment of patients with locally advanced, unresectable or metastatic pancreatic cancer.</li> </ol>	NSCLC: 150 mg/day Pancreatic cancer: 100 mg/day in combination with gemcitabine
Lapatinib	EGFR, HER2	In combination with capecitabine for the treatment of patients with advanced or metastatic breast cancer whose tumors overexpress HER2 and who have received prior therapy including an anthracycline, a taxane, and trastuzumab	1,250 mg/day on days 1-21 continuously in combination with capecitabine 2,000 mg/m <sup>2</sup> /day (administered orally in two doses approximately 12 hours apart) on days 1-14 in a repeating 21-day cycle.
Sunitinib	VEGFR-1, -2, -3, c-kit, PDGFR, CSF1-R, Flt-3, RET	<ol style="list-style-type: none"> <li>1. GIST after disease progression on or intolerance to imatinib mesylate</li> <li>2. Advanced renal cell carcinoma</li> </ol>	50 mg/day orally, with or without food, 4 weeks on treatment followed by 2 weeks off
Sorafenib	VEGFR-2, -3, c-kit, PDGFR, Raf, Flt-3, RET, FGFR-1	Advanced renal cell carcinoma	200 mg twice daily (400 mg/day)

CML, chronic myelogenous leukemia; GIST, gastrointestinal stromal tumor; ALL, acute lymphoblastic leukemia; NSCLC, non-small cell lung cancer.

[AU7]

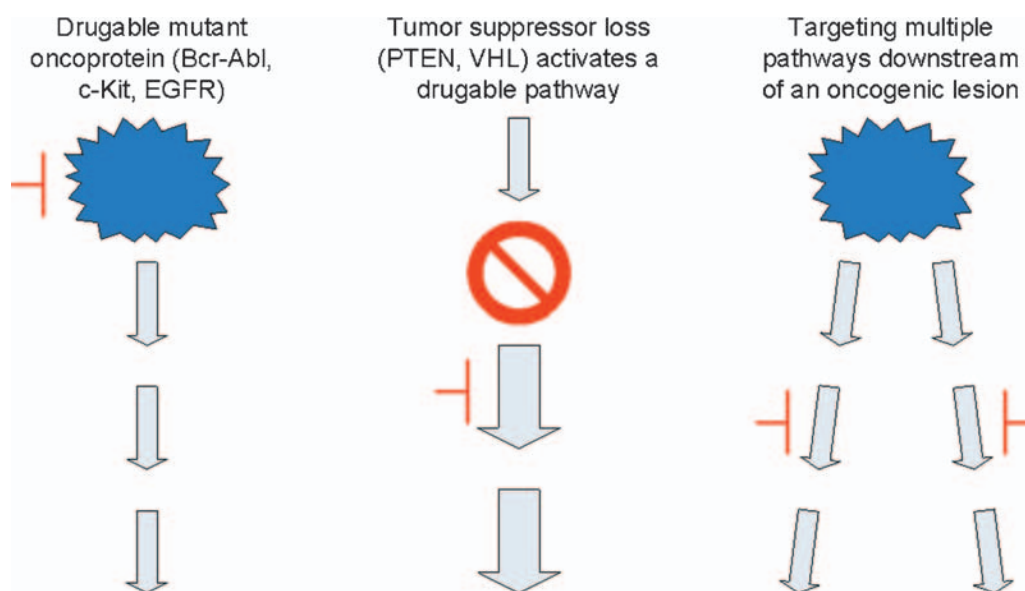
protein-folding topologies, and their common cosubstrate, ATP.<sup>6,7</sup> However, this attitude changed when the TK inhibitory properties of anilinoquinazolines were described in the mid-1990s.<sup>6</sup> Although these drugs proved to be highly selective and potent, they demonstrated poor oral bioavailability and *in vivo* exposure duration. Thus, drug developers focused on other TKIs with heightened potential for the treatment of cancer. This chapter focuses on small-molecule TKIs that have achieved United States Food and Drug Administration (FDA) approval (Table 25.9.1).

### DRUG DEVELOPMENT OF MOLECULARLY TARGETED THERAPEUTICS

There are three proposed major molecular rationales that guide targeted drug development in general and may be applied to the development of kinase inhibitors in particular (Fig. 25.9.2).<sup>8</sup> The first, or simplest model, is when the target for drug development is the oncoprotein itself. Although theoretically a simple model, the consequence of this oncopro-

tein block is typically up-regulation of downstream components of the pathway, which could increase cross-talk of multiple pathways involving those downstream targets. As a result, resistance can occur by potentially multiple venues. The classic example of the model is the TKI imatinib mesylate (Gleevec, Glivec, STI-571), which will be discussed in some detail later in this chapter. Imatinib is an inhibitor of Bcr-Abl, a mutant kinase that plays a pivotal biologic role in several disease states, most notably chronic myelogenous leukemia (CML). Inhibiting the oncoprotein target with imatinib yields therapeutic activity.

The second model is when the loss of a tumor suppressor gene can set off activation of downstream kinase cascades that can be targets of anticancer drug therapy. An example of this is the loss of the *PTEN* gene in tumors, which leads to enhanced dependence on the downstream kinase pathway, with the mammalian target of rapamycin (mTOR) serine/threonine kinase target as a specific example. Inhibiting mTOR may be an effective therapeutic venue, but recently it has been demonstrated that negative feedback loops can cause up-regulation of targets upstream from mTOR, a mechanism of resistance in this scenario.<sup>9</sup>



**Figure 25.9.2.** Three proposed major molecular rationales that guide drug development.

The final model is one in which targeting one branch of multiple pathways downstream of an oncogenic lesion may be insufficient; therefore, it may be necessary to target more than one of the downstream pathways to cause anticancer activity. In this scenario, targets for drug development within each pathway may be necessary, with either multiple agents or scientifically designing promiscuous compounds that can have an impact on multiple targets at therapeutic, achievable concentrations.

### SMALL-MOLECULE TYROSINE KINASE INHIBITORS WITH Bcr-Abl AND C-KIT AS TARGETS

The small-molecule TKI imatinib functions in a selective manner by targeting the platelet-derived growth factor receptor (PDGFR), c-kit and Abl kinases.<sup>10,11</sup> Imatinib specifically binds to the TK domain of these proteins, abrogating the binding of ATP, which is required for phosphorylation of substrate proteins, and effectively preventing the activation of downstream signaling cascades.

The fusion of the *c-Abl* gene with the *breakpoint cluster (Bcr)* gene results from the translocation of the long arms of chromosome 9 and 22 and causes the formation of the Philadelphia (Ph) chromosome.<sup>12</sup> The resulting chimeric fusion protein, Bcr-Abl, is a constitutively active protein TK present in the majority of patients with CML and 15% to 30% of adult patients with acute lymphoblastic leukemia (ALL).<sup>13</sup> Activity of Bcr-Abl has been shown to have oncogenic effects,<sup>14</sup> and the presence of the constitutively active TK is sufficient to generate the CML phenotype in normal cells.<sup>14,15</sup> Because of the relatively ubiquitous nature of the genetic abnormalities in CML patients, the disease has been an attractive focus for targeted therapy. A randomized phase 3 study of imatinib versus a combination of interferon- $\alpha$  and cytarabine in patients with newly diagnosed, chronic phase CML demonstrated a significant advantage for the TKI.<sup>16</sup> Complete hematologic response rate, major cyto-

netic response rate, major molecular response rate, and progression-free survival were all superior with imatinib treatment.<sup>16</sup> In May 2001, imatinib was approved by the FDA for the frontline treatment of CML and Ph-positive ALL and became the first commercially available TKI. Imatinib is particularly effective in the chronic phase of CML; the majority of newly diagnosed patients achieve a complete cytogenetic remission (no detectable Ph-positive cells from 20 or more bone marrow cells in metaphase) after treatment.<sup>16,17</sup> However, patients with advanced (accelerated or blast crisis) CML and Ph-positive ALL are less sensitive to imatinib, experience less frequent and transient responses, and commonly relapse within a year.<sup>17</sup>

Gastrointestinal stromal tumor (GIST) is a relatively rare tumor type that typically arises within the stomach or intestinal tract, metastasizes within the abdomen, and affects approximately 5,000 people in the United States yearly.<sup>18</sup> The development of GIST has been determined to be a result of specific mutations causing gain-of-function in the genes encoding the c-kit receptor or PDGFR $\alpha$ , found in more than 90% of patients with the disease. The overactive, uncontrolled mutant c-kit and PDGFR $\alpha$  trigger the malignant behavior of GIST tumor cells. Although GIST is highly resistant to traditional treatment with chemotherapy and radiation, imatinib therapy has also shown efficacy in patients with GIST in clinical trials. A pivotal international, randomized, phase III multicenter clinical trial determined that clinical improvement occurred in 80% of patients with advanced disease, with complete response occurring in 5% of patients, partial response in 45% to 50%, and stable disease in 25% to 30%.<sup>19</sup> In February 2002, the FDA granted approval of imatinib for the treatment of patients with c-kit-positive unresectable and/or metastatic malignant GIST.

Unfortunately, clinical refractoriness to imatinib is associated with the development of multiple mechanisms of drug resistance. In CML, resistance is conferred through *Bcr-Abl* point mutations, overexpression, and activation of selected Src family kinases.<sup>20,21</sup> In GIST, resistance is likely due to the emergence of secondary

kinase mutations in *c-kit* and *PDGFR $\alpha$* .<sup>22</sup> Resistance can be intrinsic (primary) or acquired during treatment (secondary).<sup>23</sup> Because of the occurrences of imatinib resistance, researchers have increasingly focused on the development of novel kinase inhibitors that can override imatinib resistance and bind with higher affinity to its targets.<sup>24</sup>

### DASATINIB

[AU1] Dasatinib (SPRYCEL, BM-354825) is an oral multitargeted kinase inhibitor that targets five TKs/kinase families with known involvement in cancer formation and progression: *Bcr-Abl*, *Src*, *c-Kit*, *PDGFR*, and the ephrin (EPH) receptor kinases. Dasatinib is 325-fold more potent than imatinib against cells expressing wild type *Bcr-Abl* and has demonstrated preclinical activity against 18 of 19 imatinib-resistant *Bcr-Abl* mutants.<sup>25,26</sup> Dasatinib inhibits *Bcr-Abl* by binding to both active and inactive conformations of *c-Abl*, whereas imatinib only binds to the inactive state; this difference in binding is thought to be responsible for the increased potency of dasatinib over imatinib.<sup>27</sup>

A phase 1 dose-escalating study demonstrated promising results and an acceptable safety profile for dasatinib treatment in imatinib-resistant and imatinib-intolerant patients with all phases of CML and with Ph chromosome-positive ALL.<sup>28</sup> Complete hematologic response (normal leukocyte count in peripheral blood) was noted in 93% of patients with chronic phase CML and major hematologic responses (improved but not normal leukocyte count) were seen in 70% of patients with accelerated phase CML, blast phase CML, or Ph-positive ALL.

Phase II trials have reported promising outcomes of dasatinib treatment in patients with Ph-positive ALL and at the various phases of CML. Preliminary results were recently reported from a phase 2, open-label, single-arm study of dasatinib treatment in 186 patients with imatinib-resistant or -intolerant chronic phase CML.<sup>29</sup> At follow up of 8 months, 90% of patients achieved complete hematologic responses and 52% achieved complete cytogenetic responses. Another multicenter phase 2 open-label, single-arm study of dasatinib was conducted in 107 patients with imatinib-resistant or -intolerant accelerated phase CML.<sup>30</sup> At a minimum of 8 months of follow-up, 81%, 64%, and 39% of patients achieved overall, major, and complete hematologic responses, respectively. Major and complete cytogenetic remission was achieved in 33% and 24% of patients, respectively. A similar phase 2 study in 116 patients with imatinib-resistant or -intolerant myeloid blast crisis or lymphoid blast crisis CML reported similar results in this advanced-stage population.<sup>31</sup> At the 8-month follow-up, major hematologic responses were noted in 34% of myeloid blast crisis CML patients and 31% of lymphoid blast crisis CML patients. Major cytogenetic responses were noted in 31% and 50% of these patients, respectively. Finally, a phase 2 study of 36 patients with imatinib-resistant or -intolerant Ph-positive ALL reported major hematologic responses and complete cytogenetic responses in 42% and 58% of patients, respectively.<sup>32</sup> In all four of these phase 2 studies, treatment was well tolerated and no significant differences were noted in the response to dasatinib for those patients with baseline *Bcr-Abl* point mutations known to confer imatinib resistance.

Another study examined the effects of dasatinib treatment on patients at the various phases of CML after sequential failure with both imatinib and nilotinib.<sup>33</sup> More than half (57%) of the 23 patients treated with dasatinib reported a response.

Complete hematologic responses were observed in 43% of patients and cytogenetic responses were observed in 30%. These data suggest a potential lack of cross-resistance between nilotinib and dasatinib.

Results from the various phase 2 studies of dasatinib have led to accelerated approval by the U.S. FDA for the treatment of imatinib-resistant and -intolerant CML as well as its full approval for the treatment of therapy-resistant Ph-positive ALL.<sup>34</sup> Preclinical cell studies by Shah et al.<sup>35</sup> and Schittenhelm et al.<sup>36</sup> indicate that dasatinib may also inhibit GIST-related mutations that result in resistance to imatinib. Dasatinib may therefore be useful in treating imatinib-resistant GIST, and results of current clinical studies are eagerly awaited.

### SMALL-MOLECULE TYROSINE KINASE INHIBITORS WITH EPIDERMAL GROWTH FACTOR RECEPTOR AS A TARGET

The epidermal growth factor receptor (EGFR) family comprises EGFR itself (also known as *human epidermal receptor type 1* [HER1] or ErbB1), HER2 (ErbB2), HER3 (ErbB3), and HER4 (ErbB4).<sup>37,38</sup> These TK receptors share a common molecular structure that consists of an amino-terminal extracellular domain, a single transmembrane-anchoring region, and a carboxyl-terminal intracellular domain that has TK activity. They present in the cell as inactive monomers and, upon ligand binding (from EGF, transforming growth factor- $\alpha$  [TGF- $\alpha$ ], amphiregulin, heparin-binding EGF, betacellulin, or epiregulin, among others), form homodimers or heterodimers. This results in autophosphorylation of the intracellular TK domains and activation of signaling pathways that induce several tumorigenic processes. Among the pathways activated by the EGFR is the mitogen-activated protein kinase (MAPK) pathway, which regulates gene transcription and proliferation, and the phosphatidylinositol 3, 4, 5 kinase (PI3K)/protein kinase B (PKB or Akt) signaling pathway, which mediates cell survival.

The *EGFR* signaling pathway, while present in all tissues, is activated in many tumor cells. The proportion of tumors expressing *EGFR* varies by tumor type, and may be the result of different detection methods. High levels of expression have been associated with poor outcome in many solid tumors; however, this is controversial in lung cancer despite overexpression rates of 40% to 80%.<sup>37</sup> *EGFR* signaling may be increased by a number of mechanisms besides high expression levels of *EGFR*, including receptor mutations, heterodimerization with other members of this receptor family such as *HER2* or *HER3* (*erbB2* or *erbB3*), increased expression of (autocrine/paracrine) ligands, and alterations in molecules that control receptor signaling output. Each of these activities could be assessed to give an indication of the magnitude of *EGFR* signal amplification.<sup>38</sup> Multiple ligands activating these receptors have been identified and have been considered as targets for therapeutic intervention. EGF and transforming growth factor- $\alpha$  (TGF- $\alpha$ ) bind to EGFR. However, to date no specific ligand has been identified for *HER2*.<sup>37,38</sup>

### HER2 (ErbB2)

HER2 is a member of the *EGFR* family that lacks known ligand activation and exerts its activity via heterodimerization with

[AU2]

[AU3]

other members in this family. HER2 acts as a common receptor subunit of other ErbB proteins because it enhances ligand-induced receptor activation, potentiates and prolongs the signal transduction pathways, and increases the affinity of the receptors for their ligands. The role of HER2 in patients with invasive breast cancer is well established, and is correlated with shortened disease-free survival and resistance to chemotherapy and endocrine therapy. Indeed, EGFR-HER2 or HER2-HER3 heterodimers have longer and greater proliferative signals than the corresponding homodimers. For example, synchronous overexpression of EGFR and HER2 at the protein level, which probably designates receptor heterodimerization, was detected in 10% of patients with stage I non-small cell lung cancer (NSCLC), and predicted poor outcome.<sup>39</sup>

The rationale for EGFR inhibition as a target for cancer therapy was proposed nearly 20 years ago by Mendelsohn and Baselga,<sup>38</sup> Onn et al.,<sup>39</sup> and Kawamoto et al.,<sup>40</sup> who noted that EGFR is frequently overexpressed in human tumors, and in many cases is associated with poor outcome. Hence, these tyrosine kinase receptors have resulted in multiple strategies that have been developed to target them. Approaches include monoclonal antibodies, which either bind the ligand or compete with the ligand for the extracellular domain of the receptor; inhibitors of receptor dimerization; small-molecule inhibitors of the intracellular TK domain; antisense oligonucleotides; and inhibitors of the EGFR downstream signaling network. The majority of data with small-molecule inhibitors have been in lung cancer and will be the focus on this review.

### GEFITINIB AND ERLOTINIB

Gefitinib (Iressa, ZD1839) and erlotinib (Tarceva, OSI-774) are small molecules that reversibly target EGFR TK. In early clinical trials, both produced objective responses in heavily pretreated patients with NSCLC when used as single agents.<sup>37</sup> Well absorbed after oral administration, these agents can be given for long periods of time with mild-to-moderate side effects consisting primarily of dose-dependent skin rash and diarrhea.

Two randomized phase 2 multicenter trials (Iressa Dose Evaluation in Advanced Lung Cancer, IDEAL 1 and 2) with two dosages (250 and 500 mg/day) of gefitinib were conducted in more than 400 patients with stage III or IV NSCLC whose disease had failed to respond to platinum-based chemotherapy.<sup>41,42</sup> Monotherapy with gefitinib induced radiographic response in 12% to 18% and improved symptoms in 40% to 43% of NSCLC patients with advanced disease who experienced chemotherapy failure. Side effects were generally mild, consisting of skin rash, pruritus, and diarrhea, but were significantly more common and severe at the higher dosage. In neither trial were there significant differences in efficacy variables between the 250- and 500-mg/day dosages. Hence, the recommended dosage for patients with NSCLC who have previously undergone platinum-based chemotherapy is 250 mg/day. On the basis of these data, gefitinib received approval in Japan and South Korea in July 2002 as second-line chemotherapy for advanced NSCLC, and in May 2003 in the United States as third-line monotherapy treatment of advanced disease.

Erlotinib was investigated in a phase 2 trial in 56 patients with advanced NSCLC whose disease had failed to respond to platinum-based chemotherapy.<sup>43</sup> Unlike the gefitinib studies, patients were included only if their tumors overexpressed

EGFR as defined as more than 10% positive cells. Erlotinib was given continuously at a fixed dosage of 150 mg/day, and acneiform rash was observed in 78% of patients. Response to erlotinib was reported in 12% of patients, and 39% of patients had prolonged stable disease during treatment. Gefitinib and erlotinib do not induce myelosuppression, which makes them appealing for use with chemotherapy. The results of two large, randomized, placebo-controlled, phase 3 trials (Iressa NSCLC Trial Assessing Combination Treatment [INTACT] trials 1 [gemcitabine and cisplatin] and 2 [paclitaxel and carboplatin]) of gefitinib in chemotherapy-naïve patients with stage IIIB/IV NSCLC have been reported.<sup>44,45</sup> Patients were randomly assigned to receive either placebo, gefitinib 250 mg/day, or gefitinib 500 mg/day in addition to chemotherapy. Patients continued treatment with gefitinib or placebo until disease progression occurred. Results indicated that gefitinib provided no therapeutic benefits over chemotherapy alone. In two similarly designed large, randomized trials of chemotherapy with or without erlotinib, erlotinib was given concomitantly with a combination of carboplatin and paclitaxel (the TRIBUTE study; n = 1,059) or a combination of cisplatin and gemcitabine (the TALENT study; n = 1,172).<sup>46,47</sup> Selection of patients for these trials was not based on biologic features such as *EGFR* overexpression. Like the gefitinib phase 3 trials, these studies showed that erlotinib offered no survival benefit or improvement in response rate over chemotherapy alone. In a randomized, placebo-controlled trial (NCIC BR.21) involving 731 patients, however, single-agent erlotinib was shown to prolong survival in NSCLC patients after first- or second-line chemotherapy. Overall response to erlotinib was 9%, and the overall survival durations were 6.7 months for erlotinib compared with 4.7 months for placebo ( $P = .001$ ). This was the first randomized trial to confirm that an EGFR TKI prolongs survival after first- or second-line chemotherapy. Based on these data, erlotinib received approval by the FDA in November 2004.<sup>48</sup>

Interestingly, in a similar study, gefitinib did not prolong patient survival. In the multicenter study Iressa Survival Evaluation in Lung Cancer (ISEL), 1,692 patients were randomized to receive either placebo or gefitinib 250 mg/day. This study demonstrated a difference between gefitinib and placebo in terms of survival, although this did not reach statistical significance in the overall population or in subgroups of patients.<sup>49</sup> The reasons for the differences between BR21 and ISEL results are not yet clear. Possible explanations for the discrepancy are that erlotinib was given at a dose closer to its maximal tolerated dose than gefitinib; that the study populations were not comparable (ISEL was almost twice as large), including a higher rate of refractory disease in ISEL; and that these drugs are different after all.

### PREDICTION OF EPIDERMAL GROWTH FACTOR RECEPTOR TYROSINE KINASE INHIBITOR RESPONSE

Better understanding of the biology of the EGFR system in lung cancer has been the focus of intense research in the last year. Retrospective analyses of gefitinib or erlotinib study data revealed several clinical predictors of response. They repeatedly showed that responses were more frequent among patients who had never smoked, women, patients with adenocarcinomas, and patients of East Asian ethnicity. Two pivotal studies examined

gain-of-function somatic mutations of *EGFR* in exons 18-21 and correlated them with response to EGFR inhibitors. Lynch et al.<sup>50</sup> and Paetz et al.<sup>51</sup> found increased sensitivity to gefitinib in patients with these EGFR mutations, and that patients who did not express a mutation had a low probability of responding. The group from Memorial Sloan Kettering Cancer Center extended these data and showed that similar EGFR mutations are also associated with responses to erlotinib.<sup>52</sup> These findings are supported by a report of correlation between these clinical predictors of response and EGFR mutation status. Compiling these data reveals that EGFR mutations are identified in 80% of responders to EGFR small-molecule TKI whereas mutations in *K-ras* (exon 2) are associated with lack of sensitivity to either erlotinib or gefitinib. The role of EGFR or *K-ras* mutation status in prediction of response to EGFR inhibitors in general and EGFR TKI in particular is being studied in prospective studies.<sup>53,54</sup>

Although some studies have reported that increased EGFR gene copy number is not associated with outcome of TKI therapy after measurement by quantitative polymerase chain reaction, high EGFR gene copy number measured by fluorescence *in situ* hybridization has been shown to be a predictor of a gefitinib-related effect on overall survival when compared with placebo.<sup>55</sup> Amplification of EGFR gene copy number was also demonstrated by fluorescence *in situ* hybridization analysis to be associated with responsiveness in patients treated with erlotinib.<sup>56</sup>

### MECHANISMS OF RESISTANCE TO EPIDERMAL GROWTH FACTOR RECEPTOR TYROSINE KINASE INHIBITORS

As better responses and survival to new biologic drugs continue to improve and the number of new target drugs increase, more attention is being given to the mechanism of resistance of this class of compounds. Preclinically, lung cancer cell lines that developed resistance to gefitinib presented a focal amplification of the MET proto-oncogene, by driving HER3-dependent activation of the PI3K pathway.<sup>57,58</sup> Furthermore, inhibition of MET signaling in these cells restored their sensitivity to gefitinib. An increased protein expression of vimentin combined with the loss of E-cadherin, claudin 4, and claudin 7 was associated with gefitinib resistance in both head and neck squamous cell carcinoma and NSCLC cell lines.<sup>59</sup> Accordingly, restoring E-cadherin expression increases sensitivity to EGFR inhibitors in lung cancer cell lines.<sup>60</sup> Pao et al.<sup>61</sup> showed that *K-ras* mutations *in vivo* are associated with a lack of sensitivity to gefitinib or erlotinib. The same authors described that patients whose tumors acquired resistance to gefitinib or erlotinib contain, in addition to a primary drug-sensitive mutation in *EGFR*, a secondary mutation in exon 20, which leads to substitution of methionine for threonine at position 790 (T790M) in the kinase domain.<sup>61</sup> These findings were subsequently confirmed.<sup>62</sup> Another possible mechanism of resistance in NSCLC is EGFR internalization. Drug-resistant cells showed altered receptor trafficking and demonstrated continued dependence on EGFR signaling without containing secondary *EGFR* mutations.<sup>63</sup>

Strategies to overcome resistance, either primary or acquired, can be divided into a search for compounds that target oncogenically activated kinases or drugs that inhibit downstream effectors.<sup>64</sup> A new distinct class of EGFR inhibitors, called *irreversible inhibitors*, serve as an example of the first strategy. Novel agents such as CL-387785, EKB-569, HKI-272, HKI-357,

and CI-1033 have been shown to inhibit drug-resistant mutants of EGFR.<sup>63,65</sup> Drug combinations adding different classes of drugs such as vascular endothelial growth factor receptor (VEGFR) inhibitors, histone deacetylase inhibitors, among others, to TKIs are being developed in order to effectively block downstream effectors. Ultimately, this class of agents will likely be used in selected patients based on their pathologic status.

### LAPATINIB: A DUAL KINASE INHIBITOR

Many cancer patients bear tumors that express both *EGFR* and *HER2*.<sup>66</sup> In these subjects, it is hypothesized that a compound that inhibits both targets should have significant therapeutic advantages over compounds that inhibit only one of the receptors. Lapatinib (Tykerb, GW572016) is an orally available small-molecule dual TKI that reversibly inhibits both *EGFR* and *HER2*.<sup>67,68</sup> Lapatinib acts by mimicking ATP and competes for its binding site located at the TK domain of both *EGFR* and *HER2*.<sup>69</sup> As a result of this competition, lapatinib inhibits the TK from using ATP as a cofactor for phosphorylation of tyrosine residues.<sup>69</sup>

Many clinical trials have tested lapatinib as monotherapy or in combination with other agents in a variety of cancers, including advanced breast cancer,<sup>70-72</sup> inflammatory breast cancer,<sup>73-75</sup> colorectal cancer,<sup>76</sup> ovarian cancer,<sup>77</sup> NSCLC,<sup>78</sup> bladder cancer,<sup>79</sup> hepatobiliary cancer,<sup>80</sup> head and neck cancer,<sup>81-83</sup> prostate cancer,<sup>84</sup> renal cancer,<sup>85</sup> and mixed solid tumors.<sup>86-88</sup> A phase I study (EGF10004) of lapatinib was conducted with 67 heavily pretreated patients with various metastatic solid tumors.<sup>89</sup> Patients enrolled on the study expressed *EGFR* and/or overexpressed *HER2* and were treated with doses ranging from 500 to 1,600 mg once daily. Forty-four patients (66%) experienced drug-related toxicities; however, most were mild in nature (grade 1/2, 97%). The most common drug-related adverse events were rash (31%) and diarrhea (42%), and a total of five grade 3 adverse events were reported in four patients (skin rash and gastrointestinal); no grade 4 toxicities were reported. No evidence of drug-related cardiac toxicity was observed. Four of the patients (6%) reported partial responses; all four of these had trastuzumab-resistant metastatic breast cancer, with two having inflammatory breast cancer. Twenty-four patients (36%) with other carcinomas experienced stable disease, with prolonged stable disease (6 or more months) in ten of these.

Based on the promising results of the phase I trial, studies were designed to test lapatinib in patients with metastatic breast cancer. Two combined phase 2 trials were conducted to examine the efficacy of lapatinib monotherapy in patients with *HER2*<sup>+</sup> metastatic breast cancer who had previously progressed on trastuzumab therapy.<sup>90</sup> Preliminary data from the first 81 patients enrolled on the studies indicate that a total of 19 (23%) were progression-free at 16 weeks, and 7 of them (9%) achieved either a complete or partial response.

Another phase 2 trial examined the efficacy of lapatinib monotherapy in the first-line treatment of *HER2*<sup>+</sup> metastatic breast cancer.<sup>70,91</sup> Patients were randomized to receive lapatinib at either 1,500 mg daily or 500 mg twice a day. Preliminary analysis of the first 40 patients treated indicated that there was no significant difference between the two treatment groups. No unexpected toxicities were reported, and the overall response rate of the combined groups, as assessed by independent review, was 35%.<sup>91</sup> Because of cardiac events associated with the *HER2*

inhibitor trastuzumab, cardiac safety was of particular interest on this study; left ventricular ejection fraction was determined at baseline and monitored every 8 weeks. No decreases in left ventricular ejection fraction measured more than 20% from baseline and below the lower limit of normal were noted.<sup>91,92</sup>

A pivotal multicenter phase 3 trial examined the efficacy of lapatinib combined with capecitabine.<sup>72</sup> Preclinical studies reported that capecitabine, when combined with other HER2 inhibitors, demonstrates synergistic effects.<sup>93,94</sup> Patients with progressive *HER2*<sup>+</sup> myeloid blast crisis or locally advanced breast cancer who had previously been treated with an anthracycline, a taxane, and trastuzumab, but not capecitabine, were randomized to receive either capecitabine 2,500 mg/m<sup>2</sup> daily or capecitabine 2,000 mg/m<sup>2</sup> daily plus lapatinib 1,250 mg/day for 2 weeks every 3 weeks. Median time to progression was significantly longer in the lapatinib/capecitabine arm than in the capecitabine arm (8.4 months vs. 4.4 months; hazard ratio [HR] 0.49; *P* < .001). Median progression-free survival was also significantly longer with lapatinib/capecitabine (8.4 months vs. 4.1 months; HR 0.47; *P* < .001). There was a trend toward improvement in the overall response rate (22% vs. 14%; *P* = .09) as well as fewer central nervous system metastases relapses (4 vs. 11; *P* = .10) in the 163 patients receiving lapatinib/capecitabine compared with the 161 patients receiving capecitabine alone.

Preliminary biomarker data reported at the 29th Annual San Antonio Breast Cancer Symposium indicate that the effect of lapatinib on progression-free survival may be independent of both *EGFR* tissue expression and *EGFR* extracellular domain levels.<sup>71,95</sup> In contrast, an association was noted between progression-free survival and both *HER2* tissue expression and baseline serum *HER2* extracellular domain levels, with patients with *HER2*<sup>+</sup> disease experiencing greater benefit from treatment with lapatinib compared with *HER2* patients.<sup>71</sup> This finding supports the evidence that circulating *HER2* extracellular domain levels may be a useful predictor of resistance to chemotherapy in patients with advanced breast cancer.<sup>96</sup>

Because of the promising results observed in the study, it was stopped at interim analysis and patients on the capecitabine monotherapy arm were allowed to cross over to the combination therapy arm. Based on the results of this pivotal clinical trial, the FDA, on March 13, 2007, approved the combination of lapatinib and capecitabine as second-line treatment of patients with advanced or metastatic breast cancer whose tumors overexpress *HER2* and who have received prior combination therapy including an anthracycline, a taxane, and trastuzumab.

Inflammatory breast cancer (IBC) is a particularly aggressive type of breast cancer in which the tumor cells block lymph vessels located in the skin of the breast. A phase 2 study (EGF103009) evaluated the efficacy of lapatinib in patients with recurring or refractory IBC.<sup>74,75</sup> Patients were assigned to one of two cohorts based on their expression profile of *HER2* and *EGFR*. Women who overexpressed *HER2* were placed in a single group regardless of their tumor *EGFR* status, and women whose tumors did not overexpress *HER2* but did overexpress *EGFR* were placed in the second group. All patients received lapatinib 1,500 mg/day. Of the 26 evaluable patients with *HER2*<sup>+</sup> tumors, 13 achieved a partial response or complete response for an overall response rate of 50%. In contrast, only one of 12 patients in the *HER2* cohort demonstrated response (8%). Preliminary characterization of tumor biomarkers showed that 100% of the patients who responded were positive for the phos-

phorylated (active) form of *EGFR*. Preliminary biomarker data indicate that the responders tended to overexpress *HER2*, mostly in the phosphorylated (active) form; additionally, they tended to coexpress *IGF-1R* and express activated, *p-HER-3* (*ErbB3*). Coexpression of *PTEN* deficiency did not appear to preclude the response to lapatinib in this trial. The majority of lapatinib-associated adverse events were low-grade (I/II) skin and gastrointestinal toxicities.

Another phase 2 study (EGF102580) examined the effect of the combination of lapatinib and paclitaxel as neoadjuvant therapy in patients with newly diagnosed IBC.<sup>73</sup> Treatment-naïve patients were assigned to one of two cohorts depending on their *HER2* expression status; the first cohort included only women who were *HER2*<sup>+</sup> regardless of their *EGFR* status, while the second cohort was composed of *HER2*<sup>-</sup>/*EGFR*<sup>+</sup> women. Of the 30 evaluable patients with *HER2*<sup>+</sup> tumors, 20 achieved a partial response (67%) and 3 achieved a complete response (10%), for an overall response rate of 77%. Of the 21 patients who completed surgery at data analysis, 14% of all patients or 17% of patients with *HER2* overexpressing tumors (3/18) had a complete pathologic response. In addition, 80% of the five patients whose tumors expressed *EGFR* but did not overexpress *HER2* had a partial response. These results may represent a major development in the neoadjuvant treatment of IBC, a disease that has been notoriously difficult to treat.

Currently in the early stage of enrollment, the TEACH (Tykerb Evaluation After Chemotherapy) study will be the first phase 3 clinical trial to investigate whether adjuvant treatment with lapatinib improves disease-free survival in women with early-stage *HER2*-positive breast cancer who have already completed chemotherapy treatment and are currently free of disease.<sup>95,97</sup> The women will be randomly assigned to either a single year of lapatinib monotherapy or 1 year of placebo. The TEACH study will be conducted at approximately 450 clinical research sites globally, and approximately 3,000 women who have not previously received trastuzumab will be enrolled.

ALTTO (Adjuvant Lapatinib and/or Trastuzumab Treatment Optimization) is another large-scale phase 3 study currently under design for early breast cancer patients.<sup>95</sup> The study is expected to enroll approximately 8,000 women who will be randomly assigned to one of four cohorts: trastuzumab monotherapy, lapatinib monotherapy, simultaneous combination of the two drugs, and trastuzumab and lapatinib in sequence.

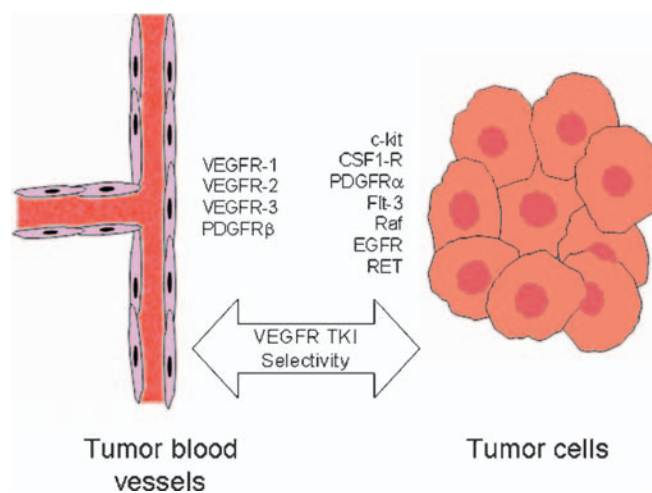
## VASCULAR ENDOTHELIAL GROWTH FACTOR RECEPTOR TYROSINE KINASE INHIBITORS

Vascular endothelial growth factor receptors (VEGFRs) are a highly conserved family of receptor TKs that control a range of intracellular processes in endothelial cells.<sup>98</sup> Expression of VEGFR-1 and VEGFR-2 is largely restricted to vascular endothelium, whereas VEGFR-3 is expressed primarily on lymphatic endothelium. VEGFR kinase activity is stimulated by binding one of five ligands (VEGF-A, -B, -C, D or PlGF), with each ligand showing a different degree of specificity for each of the receptors. In adults, VEGF-dependent signaling is a critical biologic process required for new blood vessel development in both physiological and pathologic angiogenesis. VEGF-A activation of VEGFR-2 on endothelial cells is sufficient to signal endothelial

cell proliferation, migration, and survival and is considered to be the major mediator of VEGF-dependent angiogenesis. In contrast, VEGFR-1 activation appears to provide only a weak angiogenic signal, whereas VEGFR-3 signaling plays a key role in lymphangiogenesis rather than angiogenesis. In nonendothelial cells, VEGFR-1 signaling has been shown to play a role in macrophage/monocyte migration, and VEGFR expression has been reported on tumor cells, although the functional significance of this latter observation has yet to be fully determined.

In order to continue to grow, all tumors need a blood supply in order to provide oxygen and nutrients and to get rid of waste products. Therefore, because of its pivotal role in angiogenesis, VEGFR-2 signaling has been a focus for the development of therapeutic approaches targeting new blood vessel growth in tumors. Small molecules have been designed to act as ATP-competitive kinase inhibitors targeting the ATP-binding pocket of VEGFR-2.<sup>99</sup> Because of the conserved protein structure and sequence around the ATP-binding pocket, VEGFR-2 TKIs also typically have inhibitory activity against VEGFR-1 and VEGFR-3 (Table 25.9.1). In addition, VEGFRs have strong homology to other receptor TKs, in particular the class III family of receptors,<sup>100</sup> which include *c-kit*, PDGFR $\alpha$ , PDGFR $\beta$ , Flt-3 and CSFR-1 (*c-fms*); consequently, VEGFR TKIs may have significant inhibitory activity against these receptors (Table 25.9.1). Several of these class III receptors are also relevant therapeutic targets in cancer, targeting tumor cells (*c-kit*, Flt-3, PDGFR $\alpha$ ) or tumor blood vessels (PDGFR $\beta$ ). Depending on its structure, each TKI may also have activity against receptors less closely related to the VEGFRs, including Raf, EGFR, RET, and FGFR (Table 25.9.1). Therefore, the therapeutic and toxicity profiles of each VEGFR TKI may differ significantly, depending on the balance of inhibitory activities against a range of target receptor TKs. Therefore, with VEGFR TKIs, we may anticipate a common core profile of activity/toxicity based on VEGF signaling inhibition, but with each agent distinguished by compound specific activities/toxicities based on potency and selectivity against other pharmacologic relevant receptor TKs (Fig. 25.9.3).

In preclinical models in mice, chronic dosing of VEGFR TKIs characteristically produces antitumor activity across a broad range of established histologically diverse human tumor xenografts. This broad-spectrum activity of an antiangiogenic therapeutic approach is anticipated because it targets a biologic process (VEGFR-dependent signaling) in a genetically stable cell type (endothelial cells) that is considered to be required for the continued growth of most, if not all, solid tumors. In most xenograft models, VEGFR TKIs slow or halt the growth of tumors rather than producing regressions. This is consistent with a mechanism of action that is primarily preventing new blood vessel growth, rather than destroying existing tumor vasculature or directly killing tumor cells. In addition, data from preclinical models suggest that sustained plasma exposure to VEGFR TKIs is required in order to produce long-term suppression of tumor angiogenesis and growth. The anticipated requirement for chronic dosing in the clinic has focused development of VEGFR TKIs on compounds that are suitable for oral administration, preferably once daily. In order to maintain suppression of VEGFR signaling and angiogenesis, the properties of each VEGFR TKI in terms of bioavailability, plasma protein binding, target potency, and plasma half-life need to be optimized to ensure that pharmacologically relevant plasma levels are maintained throughout the interdosing period,



**Figure 25.9.3.** The selectivity profile of small molecule vascular endothelial growth factor (VEGF) tyrosine kinase inhibitors (TKIs) will determine the balance of pharmacologic effects in the tumor vessels and tumor cells. Current VEGFR TKIs are not completely specific. Rather, in addition to inhibition of VEGFRs, these TKIs also inhibit other closely related receptors that can contribute antitumor effects through additional effects against tumor blood vessels or direct effects on tumor cell proliferation and survival. The therapeutic potential and side effect profile of small molecule VEGFR TKIs will depend on the relative selectivity and potency against each of these targets

with a relatively small ratio between the maximum and minimum plasma concentrations. Therefore, pharmaceutical properties may be as important as pharmacologic properties in discriminating the potential of VEGFR TKIs in development.

Although inhibition of VEGFR signaling alone is expected to delay tumor growth rather than produce tumor shrinkage in most solid tumor types, the additional activities that VEGFR TKIs may have against class III and other receptor TKs may have the potential to produce significant antitumor effects. Tumor-associated genetic rearrangements and/or mutations have been reported in many of the additional receptor TKs targeted by VEGFR TKIs such as *c-kit* (in GIST), PDGFR $\alpha$  (in GIST and hypereosinophilic syndrome), Flt-3 (in AML), B-Raf (serine/threonine kinase; in papillary thyroid cancer, melanoma, colorectal cancer), RET (in medullary and papillary thyroid cancer) and EGFR (in lung cancer). In preclinical models, human tumor xenografts with activating mutations in *Flt-3*, *RET*, or *EGFR* have been shown to regress when treated with VEGFR TKIs with additional activity against these receptors, indicating that these tumor targets may provide additional benefits through direct antitumor activity, particularly in those tumors that are highly dependent on these signaling pathways for growth and survival. Indeed, in patients with AML, medullary thyroid cancer or lung cancer treated with VEGFR TKIs, single-agent clinical activity has been seen in tumors harboring activating mutations in *Flt-3*, *RET*, and *EGFR* genes respectively.

Based on (i) our current biologic understanding of the roles of VEGF-signaling, (ii) preclinical models, and (iii) clinical experience with bevacizumab (an antibody directed against VEGF-A) and VEGFR TKIs, a number of adverse effects consistent with VEGF-signaling inhibition have been identified.<sup>101,102</sup>

These include hypertension, proteinuria, wound healing complications, bleeding, and reversible posterior leukoencephalopathy. Other adverse events, such as gastrointestinal perforation, thrombosis, and hypothyroidism may also be a result of VEGF signaling inhibition, although the mechanistic links have yet to be clearly determined. In contrast, adverse events such as rash, diarrhea, hand-foot syndrome, hair discoloration, and impaired cardiac function appear more likely to be the result of pharmacologic inhibition of non-VEGFR targets such as c-kit and EGFR, or the result of nonpharmacologic toxicity from the chemical nature of the TKI or its metabolites.

To date, two VEGFR TKIs (sunitinib and sorafenib) have been registered for use in cancer therapy, although many more are in early- and late-stage development.<sup>99,103,104</sup> Compared with the highly selective antibody approaches to inhibiting VEGF-signaling, sunitinib, sorafenib, and indeed many of the late-stage VEGFR TKIs are perhaps better characterized as multiple kinase inhibitors, given the range of endothelial and tumor receptor TKs potentially inhibited by these agents. As previously indicated, the efficacy/toxicity profile of each TKI is likely to be a result of both pharmacologic properties (e.g., potency, selectivity) and pharmaceutical properties (e.g., bioavailability, half-life, plasma levels).

## SUNITINIB

Sunitinib maleate (Sutent, SU11248) is indicated for the treatment of GIST in patients with disease progression on, or who do not tolerate, imatinib therapy, and it is also indicated for the treatment of advanced renal cell cancer (RCC).<sup>105,106</sup> Sunitinib is a potent inhibitor of multiple receptor TKs, including VEGFR-1, -2 and -3 (Table 25.9.1). Important tumor cell targets of sunitinib include c-kit, PDGFR $\alpha$ , and Flt-3, which are implicated in GIST and acute myeloid leukemia. Both sunitinib and its active metabolite (SU012662) have similar *in vitro* activity against VEGFR-2, c-kit, and PDGFR. Following dosing at 50 mg/day, sunitinib is rapidly absorbed (median time to C<sub>max</sub>, 8.5 hours) reaching a maximum mean plasma concentration of 72.2 ng/mL, of which 95% is protein-bound. It has been estimated that plasma levels of 50 to 100 ng/mL are required to inhibit VEGFR-2 signaling. In addition, the active metabolite SU012662 had a mean maximum plasma concentration of 33.7 ng/mL, of which 90% is protein-bound. The terminal half-life of sunitinib is 41 to 86 hours.

In preclinical models, sunitinib has been shown to inhibit the phosphorylation (activation) of VEGFR-2, Flt-3, c-kit, and PDGFR $\beta$  in human tumor xenografts, while producing significant tumor growth delays or regressions. Demonstrating inhibition of target receptors in tumors is more challenging in the clinical setting. Nonetheless, in a phase I study in acute myeloid leukemia, tumor cell phosphorylation of Flt-3 was inhibited in 50% of tumors with wild-type Flt-3, and in 100% of tumors with mutated Flt-3. The technical challenges of demonstrating inhibition of target receptors in tumor endothelium are much greater even than for tumor cells because of the low fraction of blood vessels in biopsied tissue sections (usually less than 1% or total tissue area); therefore, there has been a focus on using imaging techniques, such as dynamic contrast-enhanced magnetic resonance imaging to assess the functional tumor vasculature, or to identify circulating protein biomarkers associated with inhibition of VEGFR signaling. Both a decrease in circu-

lating VEGFR-2 and an increase in circulating VEGFR ligands (VEGF-A and PlGF) were seen in renal cancer patients during treatment with sunitinib. These changes were reversed during the 2-week period without drug, and re-established during subsequent sunitinib treatment, and the effect was seen at each cycle. Similar effects on circulating VEGFR2 and VEGF-A have also been seen in other sunitinib trials in patients with GIST or advanced malignancies, and have subsequently been reported in other clinical trials of VEGFR TKIs. These circulating proteins may represent a sensitive pharmacodynamic marker of VEGFR inhibition in patients.

The clinical efficacy of sunitinib compared with placebo has been demonstrated in a phase 3 study in patients with advanced GIST who were intolerant of imatinib, or who progressed during imatinib therapy. Compared with placebo, sunitinib treatment was associated with a significantly longer time to progression (27.3 vs. 6.4 weeks) and progression-free survival (24.1 vs. 6.0 weeks). This clinical activity may be partly because of the inhibition of c-kit TK as sunitinib may not be susceptible to the mutations that commonly confer resistance to imatinib. Data from a phase 3 randomized clinical trial investigating sunitinib compared with IFN- $\alpha$  as first-line therapy in patients with metastatic renal cell carcinoma (mRCC) demonstrated a significantly greater progression-free survival (47.3 vs. 22.0 weeks) and response rate (27.5% vs. 5.3%) for sunitinib. In two other trials as second-line treatment for mRCC patients who had progressed despite previous IFN- $\alpha$ , sunitinib demonstrated significant antitumor activity in terms of response rate (34.0% and 36.5%).

The pharmacologic activity that underlies the clinical benefit of sunitinib treatment of mRCC has not been clearly demonstrated. However, it should be noted that sunitinib is one of several VEGFR signaling inhibitors, including bevacizumab and sorafenib, which have shown activity in clinical trials in mRCC. In sporadic clear cell RCC (the most common histologic subtype), genetic changes result in nonfunctional or reduced levels of the *VHL* gene product. Loss of *VHL* leads to increased expression of VEGF and PDGF, which promote tumor angiogenesis and contribute to the highly vascular phenotype of RCC. Therefore, the activity of sunitinib and other VEGFR TKIs in RCC may be due primarily to effects on the vasculature through inhibition of VEGFR and PDGFR $\beta$ . Treatment-induced tumor shrinkage may be a result of the highly vascular nature of RCC in which VEGFR and PDGFR $\beta$  may provide survival signals to the immature blood vessels resulting in vessel regression and tumor shrinkage during treatment with sunitinib. Tumor vessel regression and tumor shrinkage have been observed in certain preclinical xenograft models of human cancer during treatment with VEGFR TKIs.

Adverse events with sunitinib were generally mild to moderate and commonly included toxicities associated with VEGFR signaling inhibitors (e.g., hypertension, bleeding), but also other toxicities of uncertain etiology (e.g., rash, myelosuppression, mucositis, hand-foot syndrome, hair and skin depigmentation, anorexia) possibly related to non-VEGFR targeted pharmacologic activities of sunitinib and its active metabolite.

Sunitinib is also being investigated in other disease settings, including breast cancer and NSCLC. However, management of the adverse event profile currently requires an intermittent dosing schedule to be used (4 weeks on 2 weeks off), perhaps making this schedule less likely to be used in combination with

standard chemotherapies in these and other disease settings. A continuous dosing schedule is under investigation.

### SORAFENIB

Sorafenib (Nexavar, BAY 43-9006) is an inhibitor of multiple receptor TKs and, in addition, inhibits C-Raf and both wild type and oncogenic B-Raf. The Raf serine/threonine kinases are the first kinases in the MAPK cascade and are critical regulators of cell proliferation and survival, acting downstream of receptor TK signaling. In terms of receptor TKs, sorafenib inhibits VEGFR-1, -2 and -3, the closely related receptors c-kit, PDGFR $\beta$ , Flt-3, and also has activity against RET, and at higher doses FGFR-1.

In preclinical models, sorafenib demonstrated broad-spectrum antitumor activity inhibiting growth in a panel of human tumor xenografts grown in nude mice with a significant reduction of tumor vessel density and inhibition of neovascularization. These studies have also demonstrated that sorafenib can inhibit signaling through the MAPK pathway through inhibition of Raf kinase activity, raising the prospect that sorafenib may inhibit at two distinct points on the same cell signaling pathway, at the receptor TK itself, and downstream at Raf/MAPK. As has been reported for other VEGF TKIs, circulating VEGFR-2 levels decreased and circulating VEGFA levels increased in renal cancer patients receiving sorafenib, providing further evidence that the changes in these proteins may provide evidence for the pharmacodynamic effects of VEGFR TKIs. In addition to its role in angiogenesis, VEGFR signaling plays an important role in blood vessel permeability. Tumor vasculature has increased permeability, which is thought to be due to increased expression of VEGF in the tumor. In preclinical models, using dynamic contrast-enhanced magnetic resonance imaging, VEGFR TKIs have been shown to reduce permeability in tumor blood vessels, and although this is not strictly a measure of VEGFR-dependent angiogenesis, it may serve as a surrogate measure of inhibition of VEGFR signaling both in the preclinical and clinical settings. In patients with metastatic renal cancer, sorafenib reduced vascular permeability and perfusion in the tumor, consistent with inhibition of VEGFR signaling. The clinical efficacy of sorafenib has been demonstrated in a randomized phase 3 study in which patients with advanced RCC who had received one prior systemic therapy were randomized to receive sorafenib or placebo. The progression-free survival for patients receiving sorafenib was 167 days compared with 84 days for patients receiving placebo. The response rates were 2% versus 0% for sorafenib and placebo, respectively. Previously, in a preclinical orthotopic renal carcinoma model in mice, sorafenib had been shown to disrupt the tumor vasculature, possibly through concurrent inhibition of both VEGFR-2 and PDGFR $\beta$  signaling in tumor blood vessels. Adverse events included diarrhea, rash, hand-foot syndrome, alopecia, hypertension, and bleeding. The latter two adverse events have been observed with a range of VEGFR TKIs in the clinic, but the spectrum of other side effects is likely to be a consequence of inhibiting the Raf-MAPK pathway, and possibly other pathways targeted by sorafenib.

Sorafenib is indicated for use in patients with advanced RCC.<sup>107,108</sup> Sorafenib is being investigated in a wide range of other disease settings both alone and in combination with chemotherapy, in clinical settings in which inhibition of Raf-MAPK signaling, VEGFR signaling, or other relevant signaling

pathways (e.g., RET) may have therapeutic potential. Ongoing studies include trials in hepatocellular carcinoma, melanoma, colorectal cancer, NSCLC, and thyroid cancer.

### OTHER VASCULAR ENDOTHELIAL GROWTH FACTOR RECEPTOR TYROSINE KINASE INHIBITORS IN CLINICAL DEVELOPMENT

A large number of additional VEGFR TKIs are at both early-stage and late-stage clinical development. Although both sunitinib and sorafenib are now FDA-approved and available to patients (Table 25.9.1), there are certain features other VEGFR TKIs may offer in terms of improved potency, selectivity, pharmacokinetics, and physical properties to achieve a more optimal balance of efficacy (inhibition of VEGFR signaling) and toxicity. In particular, chronic, uninterrupted, once-daily dosing, and the ability to combine with current, established chemotherapy regimens could be distinguishing. Typically, other than in certain exceptional cases (such as RCC), tumor responses are not anticipated with VEGFR TKIs as single agents. Therefore, as has been seen with bevacizumab, the greatest benefits of VEGF signaling inhibition may be seen as part of a combination regimen. In the future, as VEGFR TKIs move to the earlier disease settings that may be more sensitive to antiangiogenic therapies, very highly selective and potent agents targeting primarily VEGFR signaling without additional activity against class III receptors or other receptor TKs may be able to produce a tolerability profile consistent with long-term dosing while continuing to restrain new vessel growth in the tumor.

### CONCLUSION

Although TK inhibition as a target for cancer treatment has demonstrated success, there is still significant room for improvement. Progress must be made to identify mechanisms and predictors of tumor response, methods for the development of resistance, reasons for the lack of specificity of a single TKI to tumor, negative feedback, cross talk among different pathways, and horizontal and vertical bypass mechanisms, as a few examples. Significant challenges lie ahead within the next decade for medicinal chemists, clinical and basic scientists, and translational researchers involved in cancer drug development. With more than 1,500 agents in early development for the treatment of cancer, many of which are targeted against protein kinases, the drug development community is faced with the challenge of making “chemically smart” agents. Whereas multitargeted agents were once considered to be “dirty” or “promiscuous” compounds, we are now beginning to perceive the value of multitargeted inhibition in preventing undesirable events such as negative feedback and/or cross talk, and for providing therapeutic synergy through the selection of appropriate targets.

Among the many challenges facing the oncology community is the most effective appropriation of financial and patient resources to multiple agents developed against the same specific target, not only by multiple sponsors, but also by the same sponsor. These resources, the most important and valuable being the patient, are limited, and we must identify the best and most relevant leads to carry forward. With an anticipated 500 compounds entering clinical investigation within the next decade, the entire oncology community is facing this unique

challenge. How will we identify the most appropriate agents to develop clinically and how will that development ensue? Perhaps early phase 0 drug trials will prove important in navigating this maze in order to identify the most relevant anti-cancer agents to select for advanced clinical investigation.

An additional challenge that faces the drug development community is how best to evaluate response and treatment effect when determining the anticancer benefit of targeted agents. The clinical drug community is faced with how best to monitor drug response of these drugs, many of which are cytostatic and/or potentiators of other agents. Will these agents need novel measurement tools beyond RECIST (Response Evaluation Criteria in Solid Tumors)? Are routine radiographic tools the most appropriate tool for every compound?

In addition to evaluation tools and measurements, early clinical researchers must assess which trial designs and end points are most appropriate; the cancer treatment community must rethink cancer drug development and clinical trial design to properly determine the therapeutic worth of these agents. Instead of fitting a drug to a select trial design, we now have the ability to identify or develop trial designs that fit specific agents under investigation. As we move toward a more patient-specific or personalized therapy for cancer, it is critical to understand the predictive factors for each of these different TK agents and use them, alone or in combination, as early and for as long as possible (as maintenance or until progression), based on the molecular profile of a particular tumor.

## REFERENCES

- Manning G, Whyte DB, Martinez R, et al. The protein kinase complement of the human genome. *Science* 2002;298:1912.
- Steeghs N, Nortier JW, Gelderblom H. Small molecule tyrosine kinase inhibitors in the treatment of solid tumors: an update of recent developments. *Ann Surg Oncol* 2007;14:942.
- Arora A, Scholar EM. Role of tyrosine kinase inhibitors in cancer therapy. *J Pharmacol Exp Ther* 2005;315:971.
- Krause DS, Van Etten RA. Tyrosine kinases as targets for cancer therapy. *N Engl J Med* 2005;353:172.
- Madhusudan S, Ganesan TS. Tyrosine kinase inhibitors in cancer therapy. *Clin Biochem* 2004;37:618.
- Morin MJ. From oncogene to drug: development of small molecule tyrosine kinase inhibitors as anti-tumor and anti-angiogenic agents. *Oncogene* 2000;19:6574.
- Klebl BM, Daub H, Kéri G. Chemical kinomics. In: Mannhold R, Kubinyi H, Folkers G, eds. *Chemogenomics in Drug Discovery: A Medicinal Chemistry Perspective*. Weinheim: Wiley-VCH Verlag GmbH & Co., 2004:167.
- Sawyers CL. Translating a Century of Science into a Future of Cancer Prevention and Cures (Opening Plenary Session): The promise and challenges of translating molecularly targeted cancer therapy. AACR Annual Meeting; April 15, 2007
- Wan X, Harkavy B, Shen N, et al. Rapamycin induces feedback activation of Akt signaling through an IGF-1R-dependent mechanism. *Oncogene* 2007;26:1932.
- Druker BJ, Tamura S, Buchdunger E, et al. Effects of a selective inhibitor of the Abl tyrosine kinase on the growth of Bcr-Abl positive cells. *Nat Med* 1996;2:561.
- Buchdunger E, Cioffi CL, Law N, et al. 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* 2000;295:139.
- Sawyers CL. Chronic myeloid leukemia. *N Engl J Med* 1999;340:1330.
- Faderl S, Kantarjian HM, Thomas DA, et al. Outcome of Philadelphia chromosome-positive adult acute lymphoblastic leukemia. *Leuk Lymphoma* 2000;36:263.
- Lugo TG, Pendergast AM, Muller AJ, et al. Tyrosine kinase activity and transformation potency of bcr-abl oncogene products. *Science* 1990;247:1079.
- Daley GQ, Van Etten RA, Baltimore D. Induction of chronic myelogenous leukemia in mice by the P210bcr/abl gene of the Philadelphia chromosome. *Science* 1990;247:824.
- O'Brien SG, Guilhot F, Larson RA, et al. Imatinib compared with interferon and low-dose cytarabine for newly diagnosed chronic-phase chronic myeloid leukemia. *N Engl J Med* 2003;348:994.
- Druker BJ, Sawyers CL, Kantarjian H, et al. Activity of a specific inhibitor of the BCR-ABL tyrosine kinase in the blast crisis of chronic myeloid leukemia and acute lymphoblastic leukemia with the Philadelphia chromosome. *N Engl J Med* 2001;344:1038.
- Rubin BP, Heinrich MC, Corless CL. Gastrointestinal stromal tumour. *Lancet* 2007;369:1731.
- Verweij J, Casali PG, Zalcberg J, et al. Progression-free survival in gastrointestinal stromal tumours with high-dose imatinib: randomised trial. *Lancet* 2004;364:1127.
- Shah NP, Nicoll JM, Nagar B, et al. 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* 2002;2:117.
- Donato NJ, Wu JY, Stapley J, et al. BCR-ABL independence and LYN kinase overexpression in chronic myelogenous leukemia cells selected for resistance to STI571. *Blood* 2003;101:690.
- Sleijfer S, Wiemer E, Seynaeve C, et al. Improved insight into resistance mechanisms to imatinib in gastrointestinal stromal tumors: a basis for novel approaches and individualization of treatment. *Oncologist* 2007;12:719.
- Litzow MR. Imatinib resistance: obstacles and opportunities. *Arch Pathol Lab Med* 2006;130:669.
- Weisberg E, Manley P, Mestan J, et al. AMN107 (nilotinib): a novel and selective inhibitor of BCR-ABL. *Br J Cancer* 2006;94:1765.
- O'Hare T, Walters DK, Stoffregen EP, et al. In vitro activity of Bcr-Abl inhibitors AMN107 and BMS-354825 against clinically relevant imatinib-resistant Abl kinase domain mutants. *Cancer Res* 2005;65:4500.
- Shah NP, Tran C, Lee FY, et al. Overriding imatinib resistance with a novel ABL kinase inhibitor. *Science* 2004;305:399.
- Tokarski JS, Newitt JA, Chang CY, et al. The structure of Dasatinib (BMS-354825) bound to activated ABL kinase domain elucidates its inhibitory activity against imatinib-resistant ABL mutants. *Cancer Res* 2006;66:5790.
- Talpaiz M, Shah NP, Kantarjian H, et al. Dasatinib in imatinib-resistant Philadelphia chromosome-positive leukemias. *N Engl J Med* 2006;354:2531.
- Hochhaus A, Baccarani M, Sawyers C, et al. Efficacy of Dasatinib in Patients with Chronic Phase Philadelphia Chromosome-Positive CML Resistant or Intolerant to Imatinib: First Results of the CA180013 'START-C' Phase II Study. 2005;106:41. [AU4]
- Guilhot F, Apperley JF, Shah N, et al. A Phase II Study of Dasatinib in Patients with Accelerated Phase Chronic Myeloid Leukemia (CML) Who Are Resistant or Intolerant to Imatinib: First Results of the CA180005 'START-A' Study. 2005;106:39.
- Cortes J, Rousselot P, Kim DW, et al. Dasatinib induces complete hematologic and cytogenetic responses in patients with imatinib-resistant or -intolerant chronic myeloid leukemia in blast crisis. *Blood* 2007;109:3207.
- Ottmann O, Dombret H, Martinelli G, et al. Dasatinib induces rapid hematologic and cytogenetic responses in adult patients with Philadelphia chromosome-positive acute lymphoblastic leukemia with resistance or intolerance to imatinib: interim results of a Phase II study. *Blood* 2007; [AU5]
- Quintas-Cardama A, Kantarjian H, Jones D, et al. Dasatinib (BMS-354825) is active in Philadelphia chromosome-positive chronic myelogenous leukemia after imatinib and nilotinib (AMN107) therapy failure. *Blood* 2007;109:497.
- Dasatinib (Sprycel) for CML and Ph + ALL. *Med Lett Drugs Ther* 2007;49:6.
- Shah NP, Lee FY, Luo R, et al. Dasatinib (BMS-354825) inhibits KITD816V, an imatinib-resistant activating mutation that triggers neoplastic growth in most patients with systemic mastocytosis. *Blood* 2006;108:286.
- Schittenhelm MM, Shiraga S, Schroeder A, et al. Dasatinib (BMS-354825), a dual SRC/ABL kinase inhibitor, inhibits the kinase activity of wild-type, juxtamembrane, and activation loop mutant KIT isoforms associated with human malignancies. *Cancer Res* 2006;66:473.
- Pao W, Miller VA. Epidermal growth factor receptor mutations, small-molecule kinase inhibitors, and non-small-cell lung cancer: current knowledge and future directions. *J Clin Oncol* 2005;23:2556.
- Mendelsohn J and Baselga J. Status of epidermal growth factor receptor antagonists in the biology and treatment of cancer. *J Clin Oncol* 2003;21:2787.
- Onn A, Correa AM, Gilcrease M, et al. Synchronous overexpression of epidermal growth factor receptor and HER2-neu protein is a predictor of poor outcome in patients with stage I non-small cell lung cancer. *Clin Cancer Res* 2004;10:136.
- Kawamoto T, Sato JD, Le A, et al. Growth stimulation of A431 cells by epidermal growth factor: identification of high-affinity receptors for epidermal growth factor by an anti-receptor monoclonal antibody. *Proc Natl Acad Sci U S A* 1983;80:1337.
- Kris MG, Natale RB, Herbst RS, et al. Efficacy of gefitinib, an inhibitor of the epidermal growth factor receptor tyrosine kinase, in symptomatic patients with non-small cell lung cancer: a randomized trial. *JAMA* 2003;290:2149.
- Fukuoka M, Yano S, Giaccone G, et al. Multi-institutional randomized phase II trial of gefitinib for previously treated patients with advanced non-small-cell lung cancer (The IDEAL 1 Trial) [corrected]. *J Clin Oncol* 2003;21:2237.
- Perez-Soler R, Chachoua A, Hammond LA, et al. Determinants of tumor response and survival with erlotinib in patients with non-small-cell lung cancer. *J Clin Oncol* 2004; 22:3238.
- Herbst RS, Giaccone G, Schiller JH, et al. Gefitinib in combination with paclitaxel and carboplatin in advanced non-small-cell lung cancer: a phase III trial—INTACT 2. *J Clin Oncol* 2004;22:785.
- Giaccone G, Herbst RS, Manegold C, et al. Gefitinib in combination with gemcitabine and cisplatin in advanced non-small-cell lung cancer: a phase III trial—INTACT 1. *J Clin Oncol* 2004;22:777.
- Gatzemeier U, Pluzanska A, Szczesna A, et al. Results of a phase III trial of erlotinib (OSI-774) combined with cisplatin and gemcitabine (GC) chemotherapy in advanced non-small cell lung cancer (NSCLC). 2004;22:7010. [AU6]
- Herbst RS, Prager D, Hermann R, et al. TRIBUTE: a phase III trial of erlotinib hydrochloride (OSI-774) combined with carboplatin and paclitaxel chemotherapy in advanced non-small-cell lung cancer. *J Clin Oncol* 2005;23:5892.
- Shepherd FA, Rodrigues Pereira J, Ciuleanu T, et al. Erlotinib in previously treated non-small-cell lung cancer. *N Engl J Med* 2005;353:123.
- Thatcher N, Chang A, Parikh P, et al. Gefitinib plus best supportive care in previously treated patients with refractory advanced non-small-cell lung cancer: results from a randomised, placebo-controlled, multicentre study (Iressa Survival Evaluation in Lung Cancer). *Lancet* 2005;366:1527.

50. Lynch TJ, Bell DW, Sordella R, et al. Activating mutations in the epidermal growth factor receptor underlying responsiveness of non-small-cell lung cancer to gefitinib. *N Engl J Med* 2004;350:2129.
51. Paez JG, Janne PA, Lee JC, et al. EGFR mutations in lung cancer: correlation with clinical response to gefitinib therapy. *Science* 2004;304:1497.
52. Pao W, Miller V, Zakowski M, et al. EGF receptor gene mutations are common in lung cancers from "never smokers" and are associated with sensitivity of tumors to gefitinib and erlotinib. *Proc Natl Acad Sci U S A* 2004;101:13306.
53. Massarelli E, Varella-Garcia M, Tang X, et al. KRAS mutation is an important predictor of resistance to therapy with epidermal growth factor receptor tyrosine kinase inhibitors in non-small-cell lung cancer. *Clin Cancer Res* 2007;13:2890.
54. Pao W, Wang TY, Riely CJ, et al. KRAS mutations and primary resistance of lung adenocarcinomas to gefitinib or erlotinib. *PLoS Med* 2005;2:e17.
55. Hirsch FR, Varella-Garcia M, Bunn PA, Jr, et al. Molecular predictors of outcome with gefitinib in a phase III placebo-controlled study in advanced non-small-cell lung cancer. *J Clin Oncol* 2006;24:5034.
56. Tsao MS, Sakurada A, Cutz JC, et al. Erlotinib in lung cancer - molecular and clinical predictors of outcome. *N Engl J Med* 2005;353:133.
57. Engelman JA, Zejnullahu K, Mitsudomi T, et al. MET amplification leads to gefitinib resistance in lung cancer by activating ERBB3 signaling. *Science* 2007;316:1039.
58. Wang SE, Narasanna A, Perez-Torres M, et al. HER2 kinase domain mutation results in constitutive phosphorylation and activation of HER2 and resistance to EGFR tyrosine kinase inhibitors. *Cancer Cell* 2006;10:25.
59. Frederick BA, Helfrich BA, Coldren CD, et al. Epithelial to mesenchymal transition predicts gefitinib resistance in cell lines of head and neck squamous cell carcinoma and non-small cell lung carcinoma. *Mol Cancer Ther* 2007;6:1683.
60. Witte SE, Gemmill RM, Hirsch FR, et al. Restoring E-cadherin expression increases sensitivity to epidermal growth factor receptor inhibitors in lung cancer cell lines. *Cancer Res* 2006;66:944.
61. Pao W, Miller VA, Politi KA, et al. Acquired resistance of lung adenocarcinomas to gefitinib or erlotinib is associated with a second mutation in the EGFR kinase domain. *PLoS Med* 2005;2:e73.
62. Kobayashi S, Boggon TJ, Dayaram T, et al. EGFR mutation and resistance of non-small-cell lung cancer to gefitinib. *N Engl J Med* 2005;352:786.
63. Kwak EL, Sordella R, Bell DW, et al. Irreversible inhibitors of the EGF receptor may circumvent acquired resistance to gefitinib. *Proc Natl Acad Sci U S A* 2005;102:7665.
64. Rubin BP, Duenning A. Mechanisms of resistance to small molecule kinase inhibition in the treatment of solid tumors. *Lab Invest* 2006;86:981.
65. Carter TA, Wodicka LM, Shah NP, et al. Inhibition of drug-resistant mutants of ABL, KIT, and EGF receptor kinases. *Proc Natl Acad Sci U S A* 2005;102:11011.
66. Salomon DS, Brandt R, Ciardiello F, et al. Epidermal growth factor-related peptides and their receptors in human malignancies. *Crit Rev Oncol Hematol* 1995;19:183.
67. Xia W, Mullin RJ, Keith BR, et al. Anti-tumor activity of GW572016: a dual tyrosine kinase inhibitor blocks EGF activation of EGFR/erbB2 and downstream Erk1/2 and AKT pathways. *Oncogene* 2002;21:6255.
68. Rusnak DW, Lackey K, Affleck K, et al. The effects of the novel, reversible epidermal growth factor receptor/erbB-2 tyrosine kinase inhibitor, GW2016, on the growth of human normal and tumor-derived cell lines in vitro and in vivo. *Mol Cancer Ther* 2001;1:85.
69. Nelson MH, Dolder CR. Lapatinib: a novel dual tyrosine kinase inhibitor with activity in solid tumors. *Ann Pharmacother* 2006;40:261.
70. Gomez HL, Chavez MA, Doval DC, et al. A phase II, randomized trial using the small molecule tyrosine kinase inhibitor lapatinib as a first-line treatment in patients with FISH positive advanced or metastatic breast cancer. *Proc Am Soc Clin Oncol* 2005;23:Abstract 3046.
71. Cameron D, Stein S, Zaks T, et al. Lapatinib plus capecitabine shows superior efficacy compared to capecitabine alone in patients with ErbB2 positive advanced or metastatic breast cancer - initial biomarker data. *Breast Cancer Res Treat* 2006;100:Abstract 2.
72. Geyer CE, Forster J, Lindquist D, et al. Lapatinib plus capecitabine for HER2-positive advanced breast cancer. *N Engl J Med* 2006;355:2733.
73. Cristofanilli M, Boussett H, Baselga J, et al. A phase II combination study of lapatinib and paclitaxel as a neoadjuvant therapy in patients with newly diagnosed inflammatory breast cancer (IBC). *Breast Cancer Res Treat* 2006;100:Abstract 1.
74. Spector NL, Blackwell K, Hurley J, et al. EGF103009, a phase II trial of lapatinib monotherapy in patients with relapsed/refractory inflammatory breast cancer (IBC): Clinical activity and biologic predictors of response. *Proc Am Soc Clin Oncol* 2006;24:Abstract 502.
75. Trudeau M, Johnston S, Kaufman B, et al. Lapatinib (Tycerb) monotherapy in patients with recurrent inflammatory breast cancer: clinical activity and biologic predictors of response. *Ann Oncol* 2006;17 (Suppl 9): ix69, Abstract 140O.
76. Fields AL, Rinaldi DA, Henderson CA, et al. An Open-Label Multicenter Phase II Study of Oral Lapatinib (GW572016) as Single Agent, Second-Line Therapy in Patients with Metastatic Colorectal Cancer. *Proc Am Soc Clin Oncol* 2005;23:Abstract 3583.
77. Kimball KJ, Numnum TM, Estes JM, et al. A phase I trial of lapatinib in combination with carboplatin in patients with platinum sensitive recurrent epithelial ovarian cancer. *Proc Am Soc Clin Oncol* 2007;25:Abstract 14106.
78. Ross HJ, Blumenschein GR, Dowlati A, et al. Preliminary safety results of a phase II trial comparing two schedules of lapatinib (GW572016) as first line therapy for advanced or metastatic non-small cell lung cancer. *Proc Am Soc Clin Oncol* 2005;23:Abstract 7099.
79. Wülfing C, Machiels J, Richel D, et al. A single arm, multicenter, open label, phase II study of lapatinib as 2L treatment of pts with locally advanced/metastatic transitional cell carcinoma (TCC) of the urothelial tract. *Proc Am Soc Clin Oncol* 2005;23:Abstract 4594.
80. Ramanathan RK, Belani CP, Singh DA, et al. Phase II study of lapatinib, a dual inhibitor of epidermal growth factor receptor (EGFR) tyrosine kinase 1 and 2 (Her2/Neu) in patients (pts) with advanced biliary tree cancer (BTC) or hepatocellular cancer (HCC). A California Consortium (CCC-P) Trial. *Proc Am Soc Clin Oncol* 2006;24:Abstract 4010.
81. Agulnik M, Cohen EE, Cohen RB, et al. A phase II study of lapatinib in recurrent or metastatic EGFR and/or ErbB2 expressing adenoid cystic (ACC) and non-ACC malignant tumors of the salivary glands (MSGT). *Proc Am Soc Clin Oncol* 2006;24:Abstract 5566.
82. Harrington KJ, Bourhis J, Nutting CM, et al. A phase I, open-label study of lapatinib plus chemoradiation in patients with locally advanced squamous cell carcinoma of the head and neck (SCCHN). *Proc Am Soc Clin Oncol* 2006;24:Abstract 5553.
83. Abidoye OO, Cohen EE, Wong SJ, et al. A phase II study of lapatinib (GW572016) in recurrent/metastatic (R/M) squamous cell carcinoma of the head and neck (SCCHN). *Proc Am Soc Clin Oncol* 2006;24:Abstract 5568.
84. Sridhar SS, Hotte SJ, Chin JL, et al. A multicenter phase II study of lapatinib in hormone sensitive prostate cancer (HSPC). *ASCO Prostate Cancer Symposium* 2007;Abstract 261.
85. Ravaud A, Gardner J, Hawkins R, et al. Efficacy of lapatinib in patients with high tumor EGFR expression: Results of a phase III trial in advanced renal cell carcinoma (RCC). *Proc Am Soc Clin Oncol* 2006;24:Abstract 4502.
86. Dejonge M, Savage S, Verweij J, et al. A phase I, open-label study of the safety and pharmacokinetics (PK) of pazopanib (P) and lapatinib (L) administered concurrently. *Proc Am Soc Clin Oncol* 2006;24:Abstract 3088.
87. Midgley R, Flaherty KT, Haller DG, et al. Phase I study of GW572016 (lapatinib), a dual kinase inhibitor, in combination with irinotecan (IR), 5-fluorouracil (FU) and leucovorin (LV). *Proc Am Soc Clin Oncol* 2005;23:Abstract 3086.
88. Chu Q, Goldstein L, Murray N, et al. A phase I, open-label study of the safety, tolerability and pharmacokinetics of lapatinib (GW572016) in combination with letrozole in cancer patients. *Proc Am Soc Clin Oncol* 2005;23:Abstract 3001.
89. Burris HA, 3rd, Hurwitz HI, Dees EC, et al. Phase I safety, pharmacokinetics, and clinical activity study of lapatinib (GW572016), a reversible dual inhibitor of epidermal growth factor receptor tyrosine kinases, in heavily pretreated patients with metastatic carcinomas. *J Clin Oncol* 2005;23:5305.
90. Blackwell KL, Burstein H, Pegram M, et al. Determining Relevant Biomarkers from Tissue and Serum that May Predict Response to Single Agent Lapatinib in Trastuzumab Refractory Metastatic Breast Cancer. *Proc Am Soc Clin Oncol* 2005;23:Abstract 3004.
91. Gomez HL, Chavez MA, Doval DC, et al. Biomarker results from a phase II randomized study of lapatinib (GW572016) as first-line treatment for patients with ErbB2 FISH-amplified advanced or metastatic breast cancer. *Breast Cancer Res Treat* 2005;94:Abstract 1071.
92. O'Shaughnessy JA. Inhibition of the ErbB Signaling Pathway by Targeted Therapy. Presented at Translational Therapies in Breast Cancer Symposium, September 30, 2006, Boston, MA.
93. Budman DR, Soong R, Calabro A, et al. Identification of potentially useful combinations of epidermal growth factor receptor tyrosine kinase antagonists with conventional cytotoxic agents using median effect analysis. *Anticancer Drugs* 2006;17:921.
94. Magne N, Fischel JL, Dubreuil A, et al. ZD1839 (Iressa) modifies the activity of key enzymes linked to fluoropyrimidine activity: rational basis for a new combination therapy with capecitabine. *Clin Cancer Res* 2003;9:4735.
95. Tuma RS. Lapatinib moves forward in inflammatory and early HER2-positive breast cancer trials. *J Natl Cancer Inst* 2007;99:348.
96. Colomer R, Montero S, Lluch A, et al. Circulating HER2 extracellular domain and resistance to chemotherapy in advanced breast cancer. *Clin Cancer Res* 2000;6:2356.
97. Gilmer TM. Lapatinib: A novel approach to inhibit EGFR(ErbB1)/HER2(ErbB2). Presented at AACR Annual Meeting 2007.
98. Duda DG, Batchelor TT, Willett CG, et al. VEGF-targeted cancer therapy strategies: current progress, hurdles and future prospects. *Trends Mol Med* 2007;13:223.
99. Kiselyov A, Balakin KV and Tkachenko SE. VEGF/VEGFR signalling as a target for inhibiting angiogenesis. *Expert Opin Investig Drugs* 2007;16:83.
100. Grassot J, Gouy M, Perriere G, et al. Origin and molecular evolution of receptor tyrosine kinases with immunoglobulin-like domains. *Mol Biol Evol* 2006;23:1232.
101. Kamba T, McDonald DM. Mechanisms of adverse effects of anti-VEGF therapy for cancer. *Br J Cancer* 2007;96:1788.
102. Verheul HM, Pinedo HM. Possible molecular mechanisms involved in the toxicity of angiogenesis inhibition. *Nat Rev Cancer* 2007;7:475.
103. Baka S, Clamp AR, Jayson GC. A review of the latest clinical compounds to inhibit VEGF in pathological angiogenesis. *Expert Opin Ther Targets* 2006;10:867.
104. Herbst RS. Therapeutic options to target angiogenesis in human malignancies. *Expert Opin Emerg Drugs* 2006;11:635.
105. Deeks ED, Keating GM. Sunitinib. *Drugs* 2006;66:2255; discussion 2267.
106. Motzer RJ, Rini BI, Bukowski RM, et al. Sunitinib in patients with metastatic renal cell carcinoma. *JAMA* 2006;295:2516.
107. McKeage K, Wagstaff AJ. Sorafenib: in advanced renal cancer. *Drugs* 2007;67:475; discussion 484.
108. Wilhelm S, Carter C, Lynch M, et al. Discovery and development of sorafenib: a multikinase inhibitor for treating cancer. *Nat Rev Drug Discov* 2006;5:835.

[AU1]Please check/verify spelling style for gene/related terminology throughout text. Specifically, italic versus roman type, and caps versus lower case. Thank you.

[AU2]Please check throughout for cap/lower case. Previously, this was ErbB2; Gene database has an ERBB2, etc. Please make sure these are styled correctly.

[AU3]Please check for gene or protein throughout.

[AU4]Please give name of journal for refs. 29 & 30.

[AU5]Please complete ref. 32.

[AU6]AU: Please provide journal.

[AU7]Gene? Italic