Tyrosine kinase inhibitors: Why does the current process of clinical development not apply to them?

Carlos L. Arteaga[1,3,*] and Jose Baselga[2,4,*]

[1]Departments of Medicine and Cancer Biology and Breast Cancer Program, Vanderbilt-Ingram Comprehensive Cancer Center, Vanderbilt University School of Medicine, Nashville, Tennessee 37232
[2]Medical Oncology Service, Vall d'Hebron University Hospital, and Universidad Autonoma de Barcelona, Barcelona 08035, Spain
[3]Division of Oncology, Vanderbilt University Medical Center, 2220 Pierce Avenue, 777 PRB, Nashville, Tennessee 37232
[4]Medical Oncology Service, Vall d'Hebron University Hospital, P. Vall d'Hebron 119-129, Barcelona 08035, Spain
*Correspondence: carlos.arteaga@vanderbilt.edu, jbaselga@vhebron.net

The robust clinical activity of imatinib and trastuzumab for treatment of chronic myeloid leukemia, gastrointestinal stromal tumors, and breast cancer has demonstrated that blocking pathogenic tyrosine kinases can alter the natural history of human tumors. On the other hand, EGF receptor inhibitors have shown overall modest activity. The contrast in the development of these agents implies that both molecular target dependence and patient selection are essential for the successful outcome of this process. We will contrast lessons derived from the development of inhibitors of Abl, c-Kit, HER2/neu (erbB2), and EGFR, highlight successes and limitations in the field, and propose new approaches for clinical development of tyrosine kinase inhibitor therapy.

Protein tyrosine kinases catalyze the transfer of phosphate in ATP to tyrosine residues in cellular substrates. They regulate intracellular signaling pathways mediating cell proliferation, differentiation, migration, metabolism, survival, and multicellular communication. Tyrosine phosphorylation of proteins is rare and tightly regulated in quiescent cells, but abundant in rapidly proliferating or transformed cells. Intramolecular control of tyrosine kinases involves autoinhibitory mechanisms that safeguard against inappropriate or aberrant catalytic activity. Signaling pathways regulated by protein tyrosine kinases are the frequent target of somatic mutations, leading to a significant fraction of human cancers. Of the >100 dominant oncogenes known to date, many encode receptor and cytoplasmic protein tyrosine kinases known to be mutated and/or overexpressed in human cancers (Blume-Jensen and Hunter, 2001). Broad-spectrum kinase inhibitors like genistein and herbimycin have been shown to inhibit growth of cancer cells expressing activating tyrosine kinases, supporting a causal role for these enzymes in transformation. In this commentary, we will focus on Abl, c-Kit, HER2/neu (erbB2), and EGFR kinase inhibitors and critically address their therapeutic targeting in human neoplasia. The same arguments relevant to these molecules apply to serine-threonine kinase inhibitors, which are also included in the discussion.

Molecular target selection and implications for Phase I studies

Some general conditions should be considered prior to selecting a tyrosine kinase as a therapeutic target in cancer. It should be causally involved in experimental tumor progression and be identifiable in diagnostic tumor tissue. It should not have a critical role in normal postnatal or adult physiology, thus providing an exploitable "therapeutic window." Finally, basic structure/function determinants are desirable in order to develop mechanism-based inhibitors. The potential dependence of tumors on tyrosine kinase function over that of normal tissues raises several issues for their clinical development. First, these drugs are less toxic and better tolerated than conventional chemotherapy and are, therefore, potentially deliverable over a prolonged time. Second, the optimal biological dose (OBD) at which they modulate their molecular target may not match their maximally tolerated dose (MTD). Third, the kinase-dependent tumors likely to derive clinical benefit from a specific molecular inhibitor are not frequently recognizable.

The clinical development of imatinib, which inhibits the Abl, c-Kit, and PDGFR tyrosine kinases (Buchdunger et al., 2000), is a good example of how the traditional phase I, II, and III testing of kinase inhibitors does not fit the mainstream development process of anticancer chemotherapy. In the phase I (toxicity) trial of imatinib, few dose escalations were necessary prior to signs of clinical activity in patients with CML. In this trial, inhibition of both leukemic cells and Abl kinase activity correlated with a clinically active dose of imatinib (Druker et al., 2001b), which is now FDA-approved, having never reached an MTD. Another example is the phase I testing of the EGFR inhibitor gefitinib (Iressa, ZD1839). An MTD ≥700 mg/day was defined by severe rash and diarrhea (Baselga et al., 2002). However, pharmacodynamic studies in skin biopsies showed evidence of EGFR inactivation at doses ≥150 mg/day, well below the MTD (Albanell et al., 2002). Supported by these data, a phase II (efficacy) study randomized patients with advanced non-small-cell lung cancer (NSCLC) to 250 versus 500 mg/day of gefitinib. Response rate and symptom improvement were equal in both arms, but more adverse reactions were observed in patients receiving 500 mg/day (Fukuoka et al., 2003). Further, other trials with EGFR antibodies and small molecule kinase inhibitors have shown simultaneous inhibition of receptor phosphorylation in both skin and cancer cells (J. Baselga et al., 2003, Proc. Am. Soc. Clin. Oncol., abstract; J. Tabernero et al., 2003, Proc. Am. Soc. Clin. Oncol., abstract). Interestingly, the competing EGFR kinase inhibitor erlotinib (OSI-774, Tarceva) has been developed at its MTD. A recent phase III study of erlotinib given at its MTD versus best supportive care in advanced NSCLC showed improvement in survival in patients treated with the former (http://www.gene.com/gene/news/press-releases/display.do?method=detail&id=7387). Moreover, results from

phase II studies with EGFR inhibitors suggest that patients who develop a severe drug-induced rash exhibit longer survival (Perez-Soler, 2003). This last observation and the phase III study of erlotinib will prolong the debate as to whether signaling inhibitors should be developed clinically at their MTD or at their OBD.

Knowledge of the molecular or cellular target of a signaling inhibitor provides additional experimental endpoints that can lead the phase I dose-defining process. For example, inactivation of ribosomal protein S6 kinase (p70S6K) in peripheral blood mononuclear cells (PBMCs) was used to define biochemically effective doses of inhibitors of the mammalian target of rapamycin (mTOR) (Boulay et al., 2004). TOR is a serine-threonine kinase involved in response to nutrients and growth factors that activates p70S6K. Dynamic contrast-enhanced magnetic resonance imaging (DCE-MRI) has been utilized to assess tumor permeability and vascularity in patients treated with the VEGFR kinase inhibitor PTK787 (Morgan et al., 2003). The correlation between this measurement and clinical activity helped determine the OBD for PTK787, supporting the use of functional imaging for the definition of phase II doses. Finally, in transgenic prostate tumors that overexpress active Akt1, there is upregulation of genes encoding most glycoytic enzymes. Treatment of these with mTOR inhibitors downregulates RNAs for all theseenzymes (Majumder et al. 2004), suggesting that positron emission tomography (PET) that uses labeled fluorodeoxyglucose as a tracer can be utilized to define biologically effective doses of PI3K-Akt-TOR pathway inhibitors.

Imatinib (STI-571, Gleevec)

Imatinib is a small molecule that reversibly competes with ATP for binding to the kinase domain of the PDGFR, c-Kit, and Abl tyrosine kinases (Buchdunger et al., 2000). Phase I and II trials were conducted in patients with chronic phase CML, a pluripotent stem cell disorder characterized by the t(9,22) Philadelphia (Phi+) chromosome translocation. This translocation results in a fusion of Abl to the *Bcr* gene, resulting in activation of the Abl tyrosine kinase. In addition to Bcr-Abl, late phase CML contains multiple other molecular and cytogenetic abnormalities. Treatment with imatinib results in blockade of the catalytic activity of Abl and induces major hematologic remissions, with 50%–70% of patients having no evidence of Phi+ chromosome in the bone marrow after 3–6 months of therapy (Sawyers, 2003). In patients in blast crisis CML, imatinib also exhibits significant clinical activity, but responses are short-lived due to the expansion of drug-resistant CML cells (Druker et al., 2001a). Bcr-Abl gene amplification and mutations in the Abl kinase domain have been identified in patients with imatinib-resistant CML or Phi+ acute lymphoblastic leukemia (ALL) (Gorre et al., 2001). These mutations impede the binding of imatinib to the ATP pocket in Abl (Shah et al., 2002). These studies confirm the central pathogenic role of Bcr-Abl and have led to the identification of second-line inhibitors. These include dual Src/Abl kinase inhibitors capable of binding the ATP site in mutant Abl alleles and drugs that downregulate the Abl, protein such as 17-allylaminogeldanamycin (17-AAG) (Gorre et al., 2002).

The molecular "promiscuity" of imatinib has expanded its repertoire of tumor targets. Imatinib has shown remarkable activity against gastrointestinal stromal tumors (GISTs) containing activating mutations in the c-Kit tyrosine kinase. The highest activity was seen in GISTs with mutations at exons 9 and 11 of the *Kit* gene, with low activity in GISTs expressing wild-type Kit (Heinrich et al., 2003a). Interestingly, 35% of GISTs with wild-type Kit have intragenic mutations in the *PDGFRA* gene, resulting in constitutively active PDGFRα (Heinrich et al., 2003b), potentially explaining the clinical responses to imatinib in this cohort. Imatinib also exhibits robust activity against other syndromes associated with PDGFR alterations. These include chronic myelomonocytic leukemia (CMML) expressing the constitutively active TEL-PDGFRβ fusion tyrosine kinase (Apperley et al., 2002); hypereosinophilic syndrome, which contains the FIP1-L1-PDGFRα fusion protein (Cools et al., 2003); and a type of sarcoma called dermatofibrosarcoma protuberans, in which a t(17,22) chromosomal translocation leads to constitutive PDGF ligand production (Rubin et al., 2002). These data imply that activating mutations in genes encoding the molecular targets of imatinib are reliable markers of "kinase dependence" and, thus, predict for the strong clinical activity observed in single-agent trials.

Trastuzumab (Herceptin)

Trastuzumab is a humanized IgG_1 which binds to a juxtamembrane region in the HER2/neu (erbB2) receptor. HER2 is a member of the HER (erbB) family of transmembrane tyrosine kinases, which also includes the EGFR (HER1, erbB1), HER3 (erbB3), and HER4 (erbB4). Except for HER2, binding of receptor-specific ligands to the ectodomain of EGFR, HER3, and HER4 results in the formation of homodimeric and heterodimeric complexes, to which HER2 is recruited as a preferred partner (Yarden and Sliwkowski, 2001). HER2 is unable to directly interact with erbB ligands but can potently enhance signaling by HER2-containing heterodimers and/or increase the binding affinity of ligands to EGFR and HER3/4. Enhanced levels of HER2 are associated with mammary epithelial cell transformation and shorter survival in patients with breast cancer (Ross and Fletcher, 1998). Approximately 25% of invasive breast cancers exhibit HER2 gene amplification (Slamon et al., 1989). The rate of HER2 gene amplification or protein overexpression in ductal carcinoma in situ (DCIS) is the same or higher than in invasive cancers (Glockner et al., 2001), suggesting a pathogenic role for HER2 in the initiation of mammary carcinoma.

In preclinical studies, trastuzumab blocked growth of HER2-overexpresssing cells, but not cells with low receptor levels (Lewis et al., 1993). Its cellular mechanisms of action have been reviewed elsewhere, but also include inhibition of HER2 association with erbB coreceptors resulting in blockade of HER2 kinase activity (Yakes et al., 2002). Despite high controversy, the initial phase I and II studies were limited to patients with chemotherapy-refractory breast cancers, with high HER2 levels showing a response rate of approximately 20% (Cobleigh et al., 1999). Subsequently, Vogel et al. reported impressive objective response and clinical benefit rates of 35% and 48%, respectively, in patients treated with trastuzumab alone (Vogel et al., 2002). Slamon et al. reported a 25% improvement in survival in patients treated with trastuzumab plus chemotherapy versus chemotherapy alone (Slamon et al., 2001). In these studies, the above-mentioned benefit conferred by trastuzumab was limited to HER2-overexpressing cancers. Therefore, high HER2 protein levels accurately predict good odds of response to trastuzumab, which in 1998 was approved by the FDA for use in HER2-overxpressing breast cancer. The single-agent activity of this drug in metastatic breast cancers also suggests that even late, genetically complex mammary tumors remain HER2-dependent. We should emphasize that, had the pivotal trials with trastuzumab

been conducted in unselected patients, its activity would have been easily missed, thus threatening its approval by the FDA. The high likelihood of a similar risk in the development of other tyrosine kinase inhibitors cannot be underscored.

Anti-EGF receptor therapies

The success with imatinib and trastuzumab should be compared with results obtained with anti-EGFR agents (Figure 1). Trials with EGFR tyrosine kinase inhibitors in NSCLC have shown overall modest clinical activity, with higher response rates in women, nonsmokers, and patients with adenocarcinoma (Fukuoka et al., 2003; Kris et al., 2003). A detectable EGFR level in tumors was not required for study entry, in part because preclinical studies with EGFR inhibitors had demonstrated that receptor content is not a predictor of receptor utilization and hence response to treatment. In any case, no apparent attempts were made at minimally defining an EGFR-dependent population up front. Subsequently, in large phase III trials of chemotherapy ± gefitinib in NSCLC, the combination failed to improve overall survival over chemotherapy alone (Giaccone et al., 2004; Herbst et al., 2004). Similarly, the EGFR antibody cetuximab (C225, Erbitux), which inhibits receptor tyrosine kinase activity by blocking ligand-induced activation (Fan et al., 1994), failed to improve the efficacy of cisplatin-based chemotherapy over chemotherapy alone in patients with head and neck squamous cancers (B. Burtness et al., 2002, Proc. Am. Soc. Clin. Oncol., abstract).

Unlike Bcr-Abl, c-Kit, and HER2, there is little evidence to support a major pathogenic role for the wild-type EGFR in epithelial neoplasms. Therefore, the lack of selection of tumors with some evidence of EGFR-dependence is a viable explanation for the overall modest activity of gefitinib. Recent reports support this point further. First, approximately 20% of patients with high-grade CNS gliomas respond to the EGFR inhibitor erlotinib (M. Prados et al., 2003, Proc. Am. Soc. Clin. Oncol., abstract). About 40% of gliomas express the variant III (vIII) EGFR, in which an in-frame deletion in the extracellular domain results in a constitutively active mutant receptor that engages a broader spectrum of signal transducers than the wild-type EGFR (Antonyak et al., 1998). Determining the status of the *EGFR* gene in these gliomas, the ability of erlotinib to cross the blood-brain barrier, and the confounding effect of antiseizure medications taken by these patients will be required for the proper interpretation of these results. Second, two groups recently reported somatic activating mutations in the *EGFR* gene in NSCLC that exhibited durable responses to gefitinib. The mutations were either short in-frame deletions or amino acid substitutions clustered around the ATP binding pocket of the receptor tyrosine kinase domain. They were more frequent in adenocarcinoma than in other histological types of NSCLC in women and in Japanese patients (Lynch et al., 2004; Paez et al., 2004). In one of these studies, there was a suggestion that the mutant receptors were hyperresponsive to ligands, implying they confer a gain of function and, hence, EGFR dependence.

A phase III trial of erlotinib versus best supportive care in advanced NSCLC recently showed improved survival in treated patients (http://www.gene.com/gene/news/press-releases/display.do?method=detail&id=7387). It remains to be determined if the benefit from erlotinib in this trial, which was powered to achieve a 33% improvement in survival, is limited to patients with activating *EGFR* mutations. At this point, it is unknown if durable responses in the low proportion of tumors

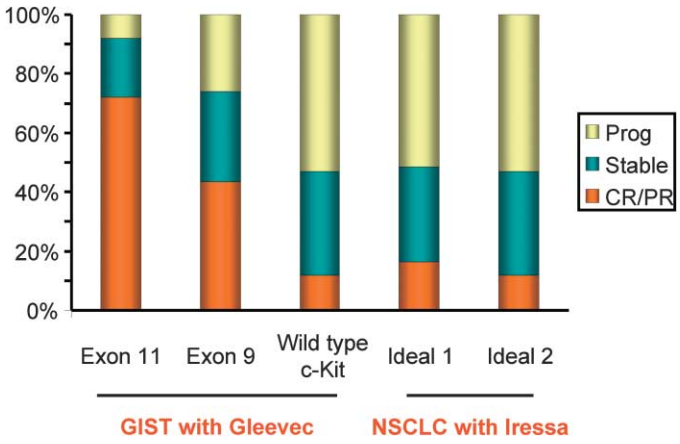

Figure 1. Clinical response rates in GIST treated with imatinib and NSCLC treated with gefitinib

There is a high response in GIST that express c-Kit with activating mutations in exons 9 and 11. The low response rate in GIST expressing wild-type Kit matches that seen in NSCLC in the IDEAL 1 and 2 single agent trials with gefitinib. The response rate in NSCLC to gefitinib approximates the recently reported EGFR mutation rate in this disease. Up to 35% of GISTs with wild-type Kit contain intragenic PDGFRα activating mutations, suggesting that in these tumors, the inhibition of the PDGFR might explain the clinical activity of imatinib. CR, complete response; PR, partial response.

bearing *EGFR* mutations can account for the improvement in overall survival. One implication from these cumulative data is that the enrollment of unselected patients into trials with EGFR inhibitors needs to be revisited in order to identify a cohort of EGFR-dependent tumors against which kinase inhibitors might exhibit clinical activity.

A strategy for patient selection into Phase II trials with kinase inhibitors

One strategy to address this issue of patient selection consists of the administration of a signaling inhibitor to patients with operable cancer immediately before definitive surgery. This presurgical approach, which can be applied to anatomically accessible cancers (i.e., breast, head and neck, colorectal), has been used extensively in breast cancer. Several studies have shown that ≤14 days of therapy with tamoxifen results in marked reduction of breast cancer proliferation as measured by Ki67 immunohistochemistry (IHC). Drug-induced inhibition of proliferation was limited to ER+ tumors (Assersohn et al., 2003), suggesting that this approach could have identified the ER as the molecular signature predictive of good odds of response to tamoxifen. This approach would also have identified ER-negative tumors as unresponsive and, therefore, point to their exclusion from phase II studies with tamoxifen. Clearly, inclusion of ER– tumors into efficacy studies would have diluted the net signal of clinical activity of the antiestrogen.

Other more recent studies suggest that measurement of drug-induced cellular activity in situ after a short time can predict clinical outcome. Chang et al. (2003, Breast Cancer Res. Treat., Suppl. 1, abstract) treated locally advanced HER2-overexpressing breast cancers with weekly trastuzumab followed by the addition of docetaxel after week 3. Significant tumor regressions at 3 weeks correlated with a 2-fold increase in tumor cell apoptosis, as measured by cleaved caspase 3 in a biopsy

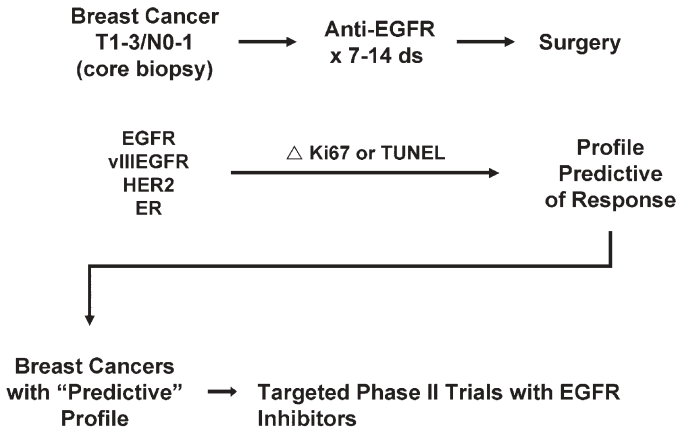

Figure 2. Exploratory trial design for molecular target validation and identification of predictive markers of response to EGFR inhibitors

Patients with operable breast cancer and patients who refuse neoadjuvant chemotherapy will be treated with the established phase II dose of an oral EGFR inhibitor for a period of ≤2 weeks. Ki67 and TUNEL will be determined in both a diagnostic core biopsy and surgical specimen to assess if there is a reduction of tumor cell proliferation and/or increase in apoptosis. A ≥75% reduction in Ki67+ cells by IHC and/or a ≥2-fold increase in apoptosis as measured by TUNEL will be then correlated with the indicated markers, all measured in paraffin-embedded tumor sections. By comparing endpoints in pre- and posttherapy specimens in this treatment-naive, in vivo model, a predictive marker(s) for response to EGFR inhibitors can be developed. This same marker(s) can be then used to select patients into targeted phase II trials that will address the clinical effect of these inhibitors. Inhibition of P-EGFR (or other marker of signaling pathway activation) in posttherapy sections is required to control for evidence of target inactivation.

obtained on day 8 after start of therapy. Further, the IMPACT trial randomized women with ER+ breast cancers to 12 weeks of neoadjuvant anastrozole, tamoxifen, or the combination. In a core biopsy obtained after 2 weeks of therapy, a statistically superior inhibition in Ki67 IHC was observed in tumors treated with anastrozole compared to the other two arms. Interestingly, this result predicted for a better efficacy of anastrozole in permitting the use of breast-conserving surgery (M. Dowsett et al., 2003, Breast Cancer Res. Treat., Suppl. 1, abstract). The results of IMPACT have additional implications for the design of phase III studies. For example, the ATAC adjuvant trial randomized >9,000 breast cancer patients to the same 3 arms but required 33 months of follow-up to reveal that anastrozole was more effective in prolonging disease-free survival (Baum et al., 2002). Based on these data, we propose that had the less expensive and shorter IMPACT trial been done first, the results of this study could have streamlined the ATAC trial by avoiding the combination arm, thus saving significant patients' time and cost.

In an ongoing trial (CLA) that aims at identifying mammary tumors with EGFR dependence, patients with newly diagnosed, operable breast cancer are treated for 7–14 days with the EGFR inhibitor erlotinib from the time of a baseline core biopsy until definitive surgery. Following surgery, the patients are subjected to the current standard of radiation, chemotherapy, and/or hormonal therapy. A flow diagram of such trial design is shown in Figure 2. Drug-induced inhibition of phospho-EGFR (or another marker of signaling pathway activation) will provide molecular evidence of target inactivation. Next, by focusing on the tumors in which the inhibitor induces an antiproliferative and/or a proapoptotic effect, it would be possible to determine a threshold level of receptors, or perhaps another molecular conventional marker(s), that correlates with cellular activity. Since all patients will be subjected to an operation as part of their standard of care, in situ cellular response data should be available in 100% of subjects enrolled. Data from this type of exploratory trial can generate a tumor profile that should assist the identification and enrollment of patients into subsequent efficacy trials with EGFR inhibitors. One plausible outcome of this study is treatment-induced loss of P-EGFR without evidence of cellular activity (as measured by Ki67 and/or TUNEL). Such negative results would anticipate lack of activity of EGFR inhibitors and, therefore, suggest that phase II trials with single-agent EGFR inhibitors in breast cancer are unwarranted. This would allow for the allocation of research resources to potentially more productive venues within the large anticancer portfolio. A similar approach can be used with other kinase inhibitors for which there is no clear molecular profile or kinase mutation to recognize kinase-dependent cancers. Perhaps more importantly, this approach could exclude patients in whom these drugs are unlikely to produce any clinical benefit.

Kinase dependence in other tumor types: Therapeutic implications

Genome sequencing approaches across tumor types has identified additional activating mutations suggestive of kinase dependence. These include B-Raf mutations in melanoma (Davies et al., 2002) and Flt3 receptor mutations in one-third of AML and a smaller group of ALL (Nakao et al., 1996). Samuels et al. identified somatic mutations in the *PI3KCA* gene, which encodes the p110α catalytic subunit of PI3K, in a diverse cohort of colon, brain, gastric, breast, and lung cancers (Samuels et al., 2004). The positions of the mutations within *PI3KCA* suggest that they may increase the enzyme's catalytic activity. The same group reported point mutations in the *KDR* gene, which encodes VEGFR2 (Bardelli et al., 2003). Although the functional consequences of KDR mutations are unclear, this result raises the possibility that the clinical activity of anti-VEGF agents and VEGFR kinase inhibitors in colorectal cancer (H. Hurwitz et al., 2003, Proc. Am. Soc. Clin. Oncol., abstract; Morgan et al., 2003) could be explained by inhibition of ligand-activated mutant KDR (VEGFR) in tumor cells.

In other cases, kinase dependence may not be associated with kinase mutation. For example, the VEGF antibody Bevacizumab (Avastin) significantly delays progression of metastatic renal cancers (Yang et al., 2003). Interestingly, 70% of nonhereditary renal cancers contain deletions or inactivating mutations of the von Hippel Lindau (*VHL*) gene, whose product encodes the ubiquitin ligase for hypoxia-inducible factor (HIF). Consequently, *VHL* mutant tumors overproduce HIF and the transcriptional targets of HIF such as PDGF, TGFα, TGFβ, and erythropoietin (George and Kaelin, 2003). Thus, the overproduction of VEGF in renal cancer indicates VEGFR kinase dependence as supported by the efficacy of anti-VEGF therapy. Based on the multiple HIF targets, we speculate that combinations of inhibitors of VEGFR, EGFR, PDGFR, and TGFβ signaling may have synergistic antitumor activity in VHL mutant cancers.

Finally, mutations in the *PTEN* tumor suppressor gene also result in kinase dependence. PTEN selectively dephosphorylates PI3,4,5P3, thus negatively regulating the product of PI3K. Loss of PTEN in a wide group of cancers (Vivanco and

Sawyers, 2002) results in increased PI3K activity and overdependence on this pathway, as PTEN null cells exhibit supersensitivity to mTOR and PI3K inhibitors (Neshat et al., 2001). Based on these studies, PTEN alterations are being used to select patients into trials with mTOR inhibitors.

Therapeutic resistance

An advantage of targeting pathogenic tyrosine kinases is that cancer cells, as a result of treatment, may modify them or their signaling partners and thus provide molecular clues about therapeutic resistance which can be used for discovery of 2nd-line strategies. One example of the strength of this approach is the demonstration that dual Abl/Src inhibitors can bind the activation loop of Abl in its closed or open conformation (Nagar et al., 2002), thus explaining their ability to inhibit CML cells expressing imatinib-resistant Abl mutations (Huron et al., 2003). In hypereosinophilic syndrome expressing the FIP1L1-PDGFRα fusion protein, acquisition of a T674I mutation is causally associated with the emergence of imatinib resistance (Cools et al., 2003). Knowledge of the structural effects of this substitution in the ATP binding pocket of the PDGFRα tyrosine kinase should help the identification of inhibitors of the resistant mutant.

In the case of trastuzumab, there are no published studies yet on primary tumors that escape antibody-induced HER2 blockade. A comprehensive effort in which tumors are rebiopsied at the time of escape will be required to gain insights as to whether this resistance is HER2-dependent or -independent and/or if it is secondary to novel *ERBB2* mutations. Drugs that might be useful in the setting of HER2-dependent resistance to trastuzumab include small molecule inhibitors of the HER2 kinase such as GW572016 (Rusnak et al., 2001), the Hsp90 inhibitor 17-AAG, which can downregulate HER2 protein levels (Basso et al., 2002), and the humanized IgG pertuzumab (2C4). 2C4 binds to an epitope different to that recognized by trastuzumab and sterically hinders the recruitment of HER2 into heregulin-induced HER2/3 heterodimers in both low and high HER2-expressing cells (Agus et al., 2002).

Conclusions

The clinical activity of imatinib and trastuzumab has proven the utility of targeting pathogenic tyrosine kinases responsible for the progression of specific cancers. On the other hand, the overall modest activity of anti-EGFR therapies suggests the need of methods that identify EGFR-dependent tumors that can be integrated into the clinical trial process. The data presented clearly indicate that kinase dependence and patient selection are central to the rational and successful development of therapeutic tyrosine kinase inhibitors. This development will require novel approaches, such as the prospective selection of patients/tumors with activating mutant kinases into trials with specific inhibitors. A second approach would be exploratory trials that examine pharmacodynamic cellular and molecular changes in tumor tissues upon inhibition of tyrosine kinases and/or a clinical trial research plan that maximizes the molecular information obtained in diagnostic tumor material.

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References

Agus, D.B., Akita, R.W., Fox, W.D., Lewis, G.D., Higgins, B., Pisacane, P.I., Lofgren, J.A., Tindell, C., Evans, D.P., Maiese, K., et al. (2002). Targeting ligand-activated ErbB2 signaling inhibits breast and prostate tumor growth. Cancer Cell *2*, 127–137.

Albanell, J., Rojo, F., Averbuch, S., Feyereislova, A., Mascaro, J.M., Herbst, R., LoRusso, P., Rischin, D., Sauleda, S., Gee, J., et al. (2002). Pharmacodynamic studies of the epidermal growth factor receptor inhibitor ZD1839 in skin from cancer patients: histopathologic and molecular consequences of receptor inhibition. J. Clin. Oncol. *20*, 110–124.

Antonyak, M.A., Moscatello, D.K., and Wong, A.J. (1998). Constitutive activation of c-Jun N-terminal kinase by a mutant epidermal growth factor receptor. J. Biol. Chem. *273*, 2817–2822.

Apperley, J.F., Gardembas, M., Melo, J.V., Russell-Jones, R., Bain, B.J., Baxter, E.J., Chase, A., Chessells, J.M., Colombat, M., Dearden, C.E., et al. (2002). Response to imatinib mesylate in patients with chronic myeloproliferative diseases with rearrangements of the platelet-derived growth factor receptor β. N. Engl. J. Med. *347*, 481–487.

Assersohn, L., Salter, J., Powles, T.J., A'Hern, R., Makris, A., Gregory, R.K., Chang, J., and Dowsett, M. (2003). Studies of the potential utility of Ki67 as a predictive molecular marker of clinical response in primary breast cancer. Breast Cancer Res. Treat. *82*, 113–123.

Bardelli, A., Parsons, D.W., Silliman, N., Ptak, J., Szabo, S., Saha, S., Markowitz, S., Willson, J.K., Parmigiani, G., Kinzler, K.W., et al. (2003). Mutational analysis of the tyrosine kinome in colorectal cancers. Science *300*, 949.

Baselga, J., Rischin, D., Ranson, M., Calvert, H., Raymond, E., Kieback, D.G., Kaye, S.B., Gianni, L., Harris, A., Bjork, T., et al. (2002). Phase I safety, pharmacokinetic, and pharmacodynamic trial of ZD1839, a selective oral epidermal growth factor receptor tyrosine kinase inhibitor, in patients with five selected solid tumor types. J. Clin. Oncol. *20*, 4292–4302.

Basso, A.D., Solit, D.B., Munster, P.N., and Rosen, N. (2002). Ansamycin antibiotics inhibit Akt activation and cyclin D expression in breast cancer cells that overexpress HER2. Oncogene *21*, 1159–1166.

Baum, M., Budzar, A.U., Cuzick, J., Forbes, J., Houghton, J.H., Klijn, J.G., and Sahmoud, T. (2002). Anastrozole alone or in combination with tamoxifen versus tamoxifen alone for adjuvant treatment of postmenopausal women with early breast cancer: First results of the ATAC randomised trial. Lancet *359*, 2131–2139.

Blume-Jensen, P., and Hunter, T. (2001). Oncogenic kinase signalling. Nature *411*, 355–365.

Boulay, A., Zumstein-Mecker, S., Stephan, C., Beuvink, I., Zilbermann, F., Haller, R., Tobler, S., Heusser, C., O'Reilly, T., Stolz, B., et al. (2004). Antitumor efficacy of intermittent treatment schedules with the rapamycin derivative RAD001 correlates with prolonged inactivation of ribosomal protein S6 kinase 1 in peripheral blood mononuclear cells. Cancer Res. *64*, 252–261.

Buchdunger, E., Cioffi, C.L., Law, N., Stover, D., Ohno-Jones, S., Druker, B.J., and Lydon, N.B. (2000). Abl protein-tyrosine kinase inhibitor STI571 inhibits in vitro signal transduction mediated by c-kit and platelet-derived growth factor receptors. J. Pharmacol. Exp. Ther. *295*, 139–145.

Cobleigh, M.A., Vogel, C.L., Tripathy, D., Robert, N.J., Scholl, S., Fehrenbacher, L., Wolter, J.M., Paton, V., Shak, S., Lieberman, G., and Slamon, D.J. (1999). Multinational study of the efficacy and safety of humanized anti-HER2 monoclonal antibody in women who have HER2-overexpressing metastatic breast cancer that has progressed after chemotherapy for metastatic disease. J. Clin. Oncol. *17*, 2639–2648.

Cools, J., DeAngelo, D.J., Gotlib, J., Stover, E.H., Legare, R.D., Cortes, J., Kutok, J., Clark, J., Galinsky, I., Griffin, J.D., et al. (2003). A tyrosine kinase created by fusion of the PDGFRA and FIP1L1 genes as a therapeutic target of imatinib in idiopathic hypereosinophilic syndrome. N. Engl. J. Med. *348*, 1201–1214.

Davies, H., Bignell, G.R., Cox, C., Stephens, P., Edkins, S., Clegg, S., Teague, J., Woffendin, H., Garnett, M.J., Bottomley, W., et al. (2002). Mutations of the BRAF gene in human cancer. Nature *417*, 949–954.

Druker, B.J., Sawyers, C.L., Kantarjian, H., Resta, D.J., Reese, S.F., Ford, J.M., Capdeville, R., and Talpaz, M. (2001a). 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. *344*, 1038–1042.

Druker, B.J., Talpaz, M., Resta, D.J., Peng, B., Buchdunger, E., Ford, J.M., Lydon, N.B., Kantarjian, H., Capdeville, R., Ohno-Jones, S., and Sawyers, C.L. (2001b). Efficacy and safety of a specific inhibitor of the BCR-ABL tyrosine kinase in chronic myeloid leukemia. N. Engl. J. Med. *344*, 1031–1037.

Fan, Z., Lu, Y., Wu, X., and Mendelsohn, J. (1994). Antibody-induced epidermal growth factor receptor dimerization mediates inhibition of autocrine proliferation of A431 squamous carcinoma cells. J. Biol. Chem. *269*, 27595–27602.

Fukuoka, M., Yano, S., Giaccone, G., Tamura, T., Nakagawa, K., Douillard, J.-Y., Nishiwaki, Y., Vansteenkiste, J., Kudoh, S., Rischin, D., et al. (2003). A multi-institutional randomized phase II trial of gefitinib for previously treated patients with advanced non-small-cell lung cancer (THE IDEAL 1 Trial). J. Clin. Oncol. *21*, 2237–2246.

George, D.J., and Kaelin, W.G., Jr. (2003). The von Hippel-Lindau protein, vascular endothelial growth factor, and kidney cancer. N. Engl. J. Med. *349*, 419–421.

Giaccone, G., Herbst, R.S., Manegold, C., Scagliotti, G., Rosell, R., Miller, V., Natale, R.B., Schiller, J.H., Von Pawel, J., Pluzanska, A., et al. (2004). Gefitinib in combination with gemcitabine and cisplatin in advanced non-small-cell lung cancer: A phase III trial–INTACT 1. J. Clin. Oncol. *22*, 777–784.

Glockner, S., Lehmann, U., Wilke, N., Kleeberger, W., Langer, F., and Kreipe, H. (2001). Amplification of growth regulatory genes in intraductal breast cancer is associated with higher nuclear grade but not with the progression to invasiveness. Lab. Invest. *81*, 565–571.

Gorre, M.E., Mohammed, M., Ellwood, K., Hsu, N., Paquette, R., Rao, P.N., and Sawyers, C.L. (2001). Clinical resistance to STI-571 cancer therapy caused by BCR-ABL gene mutation or amplification. Science *293*, 876–880.

Gorre, M.E., Ellwood-Yen, K., Chiosis, G., Rosen, N., and Sawyers, C.L. (2002). BCR-ABL point mutants isolated from patients with imatinib mesylate-resistant chronic myeloid leukemia remain sensitive to inhibitors of the BCR-ABL chaperone heat shock protein 90. Blood *100*, 3041–3044.

Heinrich, M.C., Corless, C.L., Demetri, G.D., Blanke, C.D., von Mehren, M., Joensuu, H., McGreevey, L.S., Chen, C.J., Van den Abbeele, A.D., Druker, B.J., et al. (2003a). Kinase mutations and imatinib response in patients with metastatic gastrointestinal stromal tumor. J. Clin. Oncol. *21*, 4342–4349.

Heinrich, M.C., Corless, C.L., Duensing, A., McGreevey, L., Chen, C.J., Joseph, N., Singer, S., Griffith, D.J., Haley, A., Town, A., et al. (2003b). PDGFRA activating mutations in gastrointestinal stromal tumors. Science *299*, 708–710.

Herbst, R.S., Giaccone, G., Schiller, J.H., Natale, R.B., Miller, V., Manegold, C., Scagliotti, G., Rosell, R., Oliff, I., Reeves, J.A., et al. (2004). Gefitinib in combination with paclitaxel and carboplatin in advanced non-small-cell lung cancer: A phase III trial–INTACT 2. J. Clin. Oncol. *22*, 785–794.

Huron, D.R., Gorre, M.E., Kraker, A.J., Sawyers, C.L., Rosen, N., and Moasser, M.M. (2003). A novel pyridopyrimidine inhibitor of abl kinase is a picomolar inhibitor of Bcr-abl-driven K562 cells and is effective against STI571-resistant Bcr-abl mutants. Clin. Cancer Res. *9*, 1267–1273.

Kris, M.G., Natale, R.B., Herbst, R.S., Lynch, T.J., Jr., Prager, D., Belani, C.P., Schiller, J.H., Kelly, K., Spiridonidis, H., Sandler, A., et al. (2003). 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 *290*, 2149–2158.

Lewis, G.D., Figari, I., Fendly, B., Wong, W.L., Carter, P., Gorman, C., and Shepard, H.M. (1993). Differential responses of human tumor cell lines to anti-p185HER2 monoclonal antibodies. Cancer Immunol. Immunother. *37*, 255–263.

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

Majumder, P.K., Febbo, P.G., Bikoff, R., Berger, R., Xue, Q., McMahon, L.M., Manola, J., Brugarolas, J., McDonnell, T.J., Golub, T.R., et al. (2004). mTOR inhibition reverses Akt-dependent prostate intraepithelial neoplasia via regulation of apoptotic and Hif1-dependent pathways. Nat. Med. Published online May 23, 2004. 10.1038/nm1052.

Morgan, B., Thomas, A.L., Drevs, J., Hennig, J., Buchert, M., Jivan, A., Horsfield, M.A., Mross, K., Ball, H.A., Lee, L., et al. (2003). Dynamic contrast-enhanced magnetic resonance imaging as a biomarker for the pharmacological response of PTK787/ZK 222584, an inhibitor of the vascular endothelial growth factor receptor tyrosine kinases, in patients with advanced colorectal cancer and liver metastases: Results from two phase I studies. J. Clin. Oncol. *21*, 3955–3964.

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

Nakao, M., Yokota, S., Iwai, T., Kaneko, H., Horiike, S., Kashima, K., Sonoda, Y., Fujimoto, T., and Misawa, S. (1996). Internal tandem duplication of the flt3 gene found in acute myeloid leukemia. Leukemia *10*, 1911–1918.

Neshat, M.S., Mellinghoff, I.K., Tran, C., Stiles, B., Thomas, G., Petersen, R., Frost, P., Gibbons, J.J., Wu, H., and Sawyers, C.L. (2001). Enhanced sensitivity of PTEN-deficient tumors to inhibition of FRAP/mTOR. Proc. Natl. Acad. Sci. USA *98*, 10314–10319.

Paez, J.G., Janne, P.A., Lee, J.C., Tracy, S., Greulich, H., Gabriel, S., Herman, P., Kaye, F.J., Lindeman, N., Boggon, T.J., et al. (2004). EGFR mutations in lung cancer: Correlation with clinical response to Gefitinib therapy. Science. Published online April 29, 2004. 10.1126/science.1099314.

Perez-Soler, R. (2003). Can rash associated with HER1/EGFR inhibition be used as a marker of treatment outcome? Oncology *17*, 23–28.

Ross, J.S., and Fletcher, J.A. (1998). The HER-2/neu oncogene in breast cancer: prognostic factor, predictive factor, and target for therapy. Stem Cells *16*, 413–428.

Rubin, B.P., Schuetze, S.M., Eary, J.F., Norwood, T.H., Mirza, S., Conrad, E.U., and Bruckner, J.D. (2002). Molecular targeting of platelet-derived growth factor B by imatinib mesylate in a patient with metastatic dermatofibrosarcoma protuberans. J. Clin. Oncol. *20*, 3586–3591.

Rusnak, D.W., Lackey, K., Affleck, K., Wood, E.R., Alligood, K.J., Rhodes, N., Keith, B.R., Murray, D.M., Knight, W.B., Mullin, R.J., and Gilmer, T.M. (2001). 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. *1*, 85–94.

Samuels, Y., Wang, Z., Bardelli, A., Silliman, N., Ptak, J., Szabo, S., Yan, H., Gazdar, A., Powell, S.M., Riggins, G.J., et al. (2004). High frequency of mutations of the PIK3CA gene in human cancers. Science *304*, 554.

Sawyers, C.L. (2003). Opportunities and challenges in the development of kinase inhibitor therapy for cancer. Genes Dev. *17*, 2998–3010.

Shah, N.P., Nicoll, J.M., Nagar, B., Gorre, M.E., Paquette, R.L., Kuriyan, J., and Sawyers, C.L. (2002). Multiple BCR-ABL kinase domain mutations confer polyclonal resistance to the tyrosine kinase inhibitor imatinib (STI571) in chronic phase and blast crisis chronic myeloid leukemia. Cancer Cell *2*, 117–125.

Slamon, D.J., Godolphin, W., Jones, L.A., Holt, J.A., Wong, S.G., Keith, D.E., Levin, W.J., Stuart, S.G., Udove, J., Ullrich, A., et al. (1989). Studies of the HER-2/neu proto-oncogene in human breast and ovarian cancer. Science *244*, 707–712.

Slamon, D.J., Leyland-Jones, B., Shak, S., Fuchs, H., Paton, V., Bajamonde, A., Fleming, T., Eiermann, W., Wolter, J., Pegram, M., et al. (2001). Use of chemotherapy plus a monoclonal antibody against HER2 for metastatic breast cancer that overexpresses HER2. N. Engl. J. Med. *344*, 783–792.

Vivanco, I., and Sawyers, C.L. (2002). The phosphatidylinositol 3-Kinase AKT pathway in human cancer. Nat. Rev. Cancer *2*, 489–501.

Vogel, C.L., Cobleigh, M.A., Tripathy, D., Gutheil, J.C., Harris, L.N., Fehrenbacher, L., Slamon, D.J., Murphy, M., Novotny, W.F., Burchmore, M., et al. (2002). Efficacy and safety of trastuzumab as a single agent in first-line

treatment of HER2-overexpressing metastatic breast cancer. J. Clin. Oncol. *20*, 719–726.

Yakes, F.M., Chinratanalab, W., Ritter, C.A., King, W., Seelig, S., and Arteaga, C.L. (2002). Herceptin-induced inhibition of phosphatidylinositol-3 kinase and Akt Is required for antibody-mediated effects on p27, cyclin D1, and antitumor action. Cancer Res. *62*, 4132–4141.

Yang, J.C., Haworth, L., Sherry, R.M., Hwu, P., Schwartzentruber, D.J., Topalian, S.L., Steinberg, S.M., Chen, H.X., and Rosenberg, S.A. (2003). A randomized trial of bevacizumab, an anti-vascular endothelial growth factor antibody, for metastatic renal cancer. N. Engl. J. Med. *349*, 427–434.

Yarden, Y., and Sliwkowski, M.X. (2001). Untangling the ErbB signalling network. Nat. Rev. Mol. Cell Biol. *2*, 127–137.