Targeting protein kinases in cancer therapy: a success?

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The fundamental role of kinases in cancer progression has promoted the development of a plethora of therapeutic inhibitors. Despite the promise of effective treatment with little associated toxicity, the clinical experience with these agents has been mixed. This review will summarize recent advances made in the development of kinase inhibitors to highlight emerging issues and the strategies by which they being addressed.

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Rationale for targeting kinases in cancer therapy

Many of the defining characteristics of cancer, including uncontrolled growth, survival, neovascularization, metastasis and invasion, result from perturbation of regulatory signaling pathways, which are normally under tight control. As advances are made in understanding the mechanisms underlying the development of cancer, it has become clear that particular pathways are more frequently deregulated. Deregulation, whether as a result of deletion, mutation or amplification of component gene products, is manifested as aberrant activation of key regulators of these pathways, a prime example of which are kinases [1]. A molecular targeted therapy that specifically inhibits such pivotal regulators would be expected to bypass the toxicity associated with currently used chemotherapeutic agents. This possibility has spurred intense activity within the pharmaceutical industry into the development of agents directed against protein kinases. Currently, over 20 different kinases, the majority being receptor tyrosine kinases (RTKs), are being considered as potential therapeutic targets in oncology.

Although the success of imatinib and trastuzumab has provided a proof of concept that such agents can be both therapeutically effective and retain an acceptable safety profile, the clinical experience with many tyrosine kinase inhibitors has been less promising. This review is not intended as a general overview of the role of kinases in cancer or kinase inhibitors currently in the clinic, but instead will focus on the emerging criteria that should be met for a kinase inhibitor to be successful.

Biology & target validation

Understanding the role of a potential target in cancer development and progression is as relevant as the efficient optimization of an inhibitory compound's potency, toxicity and pharmacokinetic profile. To be a valid target, a kinase should play a fundamental role in the pathogenesis of the disease. Activating mutations have been used as a rationale to determine potential kinase and disease targets in oncology. Genetic screening of families has linked germline kinase mutations, which lead to a gain of function, with a predisposition to cancer, for example, the association of mutations in c-Met with familial papillary renal cell carcinoma or multiple endocrine neoplasia Type 2 in families with c-Ret mutations [2], or gastrointestinal stromal tumor (GIST) with activating c-Kit mutations [3]. The presence of identical mutations in sporadic versions of the same cancer further reinforces the hypothesis of a fundamental role in progression of the disease. Increased understanding of the molecular history of the disease is also invaluable. Mutations that are found to be expressed in early phases of the disease, such as those found within c-Kit in GIST or the Philadelphia chromosome translocation in chronic phase chronic myeloid leukemia (CML), are more likely to drive disease progression than those that occur later. A correlation between

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the metastasis/aggressiveness of a type of cancer and a particular mutation has also been used to validate a candidate kinase, for example, epidermal growth factor receptor (EGFR)vIII mutants in glioblastoma [4]. Accumulating evidence suggests that the prevalence of kinase mutations in solid tumors is far greater than previously thought. A large-scale genomics sequencing approach identified an unexpectedly high frequency of BRAF mutations in melanoma [5]. In addition, point mutations in several tyrosine kinases and within the phosphatidylinositol 3-kinase catalytic subunit (PI3KCA) have been identified in colorectal cancer. Mutations in PI3KCA, that resulted in higher kinase activity, were also found to occur within several other cancer types, including glioblastoma and gastric cancer [6,7].

In the absence of information on kinase mutational status, overexpression of a kinase, particularly RTKs, has been used as a rationale to design inhibitors, for example, EGFR-targeting agents such as gefitinib and erlotinib. However, it is worth noting that a low (≤10%) percentage of patients responded to treatment with these agents and this response has been linked to the presence of novel mutations in the EGFR [8,9]. Despite this, it is clear that kinase inhibitors can be clinically effective in situations where the kinase is not mutated (e.g., treatment of patients with breast cancer overexpressing HER-2 with trastuzumab or dermatofibrosarcoma protuberans with imatinib) [10,11]. One of the current priorities in kinase-centric cancer research is therefore the identification of additional factors, other than solely expression levels, that delineate whether a tumor is dependent on a particular kinase. Approaches can include the use of phosphoproteomic or gene expression profiling analysis, examples of the latter being used to identify a kinase transformation fingerprint in human colorectal cancer and to define different prognoses, and efficacy of treatment has already been reported [12–14].

Tumors are dependent on neovascularization, the development of new blood vessels, to maintain a sufficient supply of nutrients and oxygen, which has led to the design of inhibitors directed against kinases that regulate this process. The clinical success of bevacizumab, an antibody that inhibits vascular endothelial growth factor receptor (VEGFR) signaling, has provided proof of concept for this approach [15]; however, additional targets on endothelial cells and other components of the vascular architecture are also being investigated.

Selectivity & approaches

The successful completion of the human genome has led to the advent of the kinome, currently containing more than 520 members [16]. It is apparent that any successful inhibitor should specifically target the intended kinase without affecting closely related subfamily members. The preponderance of RTKs as cancer targets has facilitated the development of therapeutic antibodies that inhibit receptor activation, either by preventing ligand–receptor interaction or by binding the receptor itself, inducing receptor internalization and, in parallel, causing cell killing via an antibody-dependent cellular cytotoxicity (ADCC) response. Genetic engineering has enabled the development of fully humanized antibodies that overcome potential antiantibody reactions and increase clinical efficacy. In a further level of sophistication, bispecific antibodies that recognize two different antigens have been developed. For example, MDX-447 and MDX-H210 (Medarex) contain one domain that recognizes either EGFR or HER-2, respectively, and another that is specific for CD64, thereby directing cytotoxic effector cells to receptor-overexpressing target cells. MDX-447 has entered Phase II clinical trials for the treatment of head and neck cancers that overexpress EGFR [17–19]. Some of the antibodies currently in clinical development are presented in TABLE 1.

Clinical agents derived from naturally occurring compounds comprise those directed towards the ATP-binding site and those with alternative mechanisms of action. Examples include flavopiridol (FPD/L868275/HMR1275), a semisynthetic flavone that associates with the ATP-binding site of cyclin-dependent kinases (CDKs) [20], and derivatives of the macrolide antibiotic rapamycin (sirolimus/Rapamune®), CCI-779 and Rad001 (everolimus/Certican®) that complex with the 12-kDa immunophilin FK506-binding protein (FKBP)-12, thereby specifically inhibiting the target of rapamycin (TOR) serine/threonine kinase [21].

In contrast, rationally designed low-molecular-weight compounds currently in the clinic are almost exclusively directed against the ATP-binding site of the kinase. ATP interacts, predominantly, via lipophilic/van der Waals interactions, within a cleft formed between two lobes of the kinase. Although it was initially felt that the conserved nature of the kinase catalytic domain would render identification of specific inhibitors difficult, alternative strategies, including attempts to design compounds that abrogate receptor–ligand interaction or prevent phosphotyrosine–Src homology (SH)-2 domain binding, have not met with clinical success. Cocrystallization of kinases with ATP analogs or inhibitors, as well as homology modeling studies, have been used to identify key structural features of ATP-binding sites that permit the design of relatively specific inhibitors [22]. As it appears improbable that completely specific inhibitors will be developed (i.e., one inhibitor, one kinase), attention has been focused on obtaining therapeutically beneficial clinical use in the absence of limiting toxicities.

Another method to obtain specificity within the ATP-binding site has been to target the inactive conformation of a kinase. Crystallization studies of different kinases have suggested that, whereas the active 3D conformation may be constrained due to catalytic necessity, greater diversity is observed in the structure of the inactive conformation – a phenomenon that presumably also reflects the diverse mechanisms by which kinase activity needs to be biologically regulated [23]. PD-173955 is a more potent but less specific inhibitor of Abl than imatinib. Solving the crystal structure of Abl in complex with either inhibitor suggests that this is due to the fact that PD-173955 is able to interact with multiple (active and inactive) conformations of the kinase, whereas imatinib specifically freezes the kinase into

Table 1. Examples of antibodies currently in clinical development.

Agent	Company	Structure	Target
Cetuximab (IMC-225)	ImClone/Merck/ Bristol–Myers Squibb	Chimeric monoclonal	EGFR
Trastuzumab	Genentech	Humanized monoclonal	HER-2
Pertuzumab	Genentech	Humanized monoclonal	HER-2
MDX-447	Medarex/Merck	Humanized bivalent monoclonal	EGFR
MDX-H210	Medarex/Novartis	Humanized bivalent monoclonal	HER-2
Bevacizumab	Genentech	Humanized monoclonal	VEGF
IMC-1C11	ImClone	Chimeric monoclonal	VEGFR2

EGFR: Epidermal growth factor receptor; VEGF(R): Vacular endothelial growth factor (receptor).

an inactive conformation. As discussed in the following section, one consequence of the specificity gained by imatinib binding the inactive kinase conformation is the risk of emergence of point mutations that can convey resistance to the drug without compromising catalytic activity. It is predicted that resistance mutations would be less likely to arise with compounds directed against the active conformation, as these would also perturb the catalytically active 3D kinase structure [24]. A representative sample of kinase inhibitors currently in the clinic is provided in TABLE 2.

Clinical examples of kinase inhibitors

Imatinib

The Philadelphia chromosome, which results in fusion and constitutive activation of the intracellular kinase Abl with Bcr (Bcr-Abl), is present in 95% of patients with CML and 15–30% of patients with acute lymphoblastic leukemia (ALL). Bcr-Abl is the unique cytogenetic aberration in chronic phase CML and induces a CML-like disease when expressed in murine bone marrow [25]. Imatinib, a potent inhibitor of Abl, platelet-derived growth factor receptor (PDGFR) and c-Kit, blocked Bcr-Abl-mediated proliferation of established cell lines and freshly isolated CML and ALL blasts [26,27]. Clinical response of patients at all stages of CML resulted in the approval of imatinib by the US Food and Drug Administration (FDA) for treatment of this indication [27,28]. Subsequently, imatinib was also approved for the treatment of GIST, a rare, chemoresistant sarcoma [29,30]. Imatinib is clinically effective in GIST due to the expression of constitutively activated c-Kit or, more infrequently, PDGFRα mutants [31,32]. Subsequently, other malignancies that display constitutive activation of the PDGFR, either due to the presence of aberrantly expressed ligand, the COL1A1-PDGFB fusion in dermatofibrosarcoma protuberans [33,34], or fusion of the receptor TEL-PDGFR in chronic myelomonocytic leukemia (CMLL) [35] and Fip1L1-PDGFRα in hypereosinophilic syndrome (HES), have responded to treatment with imatinib [36]. Imatinib is additionally being considered for treatment of other neoplasms characterized by the presence of c-Kit mutants, such as seminoma [37] and systemic mast cell disease [38].

Issues in the clinic: emergence of resistance

Notably, although a stable response has been observed in chronic phase CML patients, most patients in accelerated or blast crisis, after an initial response, developed resistance to imatinib and relapsed [39]. In a similar manner, initial response of HES and GIST patients has been followed by the emergence of imatinib-resistant disease [40,41]. It has been suggested that resistance is an inevitable consequence of the treatment of genetically unstable disease with a single molecularly targeted agent. Several nonexclusive mechanisms by which resistance could occur can be envisaged: target amplification; mutation of the target to prevent action of the inhibitor; activation of a complementary pathway that bypasses requirement for the target; and upregulation of mechanisms that lower the intracellular concentrations of the inhibitor. However, analysis of CML patients, who have relapsed on treatment with imatinib, indicated that at least half of relapsed patients display point mutations within the kinase domain of Bcr-Abl [42,43]. Experimental recapitulation of these mutations has demonstrated that, to a greater or lesser extent, they render kinase activity resistant to the effects of imatinib [44]. The detection of mutations in two untreated blast phase CML patients, who subsequently failed to respond to imatinib, suggests that these mutations were not generated by exposure to the drug. Instead, it appears likely that a population of CML blasts contains multiple, albeit rare, mutant clones that are expanded by virtue of the resistance of their catalytic activity to the presence of imatinib [45,46]. The isolation of Fip1L1-PDGFR containing a Thr674Ile mutation, analogous with Thr315Ile imatinib-resistant mutation observed in Bcr-Abl, from an HES patient who relapsed after treatment with drug, suggests that the appearance of resistance mutations is not a peculiarity of Bcr-Abl or CML patients [36].

As described previously, imatinib binds to an inactive conformation of Abl, fitting in a pocket normally occupied by the DFG activation loop in the catalytically active kinase conformation [47,48]. After the initial finding of the Thr315Ile mutation, reported by Gorre and coworkers, over 30 different mutations have been identified in imatinib-resistant patients. Mapping the drug-resistant point mutations suggests that whereas some affect residues that make direct contact with imatinib, others convey resistance by sterically hindering the ability of the kinase to achieve an inactive conformation [46].

Experimental mutagenesis of Bcr-Abl kinase was used to identify amino-acid substitutions that conveyed resistance to imatinib [49]. Over 90 different mutations were identified, including all of the

Table 2. Examples of small-molecule kinase inhibitors in clinical development.

Agent	Company	Target	Indication
Gefitinib	AstraZeneca	EGFR	NSCLC
Erlotinib	OSI/Genentech/Roche	EGFR	NSCLC
Valatanib (PTK787/ ZK222584)	Novartis/Schering AG	VEGFR	Solid tumors
SU11248	Pfizer	VEGFR c-Kit Flt-3	Solid tumors GIST AML
Imatinib	Novartis	ABL PDGFR c-Kit	CML CMML, HES GIST
BMS-354825	Bristol–Myers Squibb	Src, Abl	Solid tumors
Rapamycin/ CCI-779	Wyeth	TOR	Solid tumors
SU011248	SUGEN/Pfizer	PDGF-R, Flt3, c-Kit, VEGFR	GIST
RAD001	Novartis	TOR	Solid tumors
Midostaurin/ PKC412	Novartis	Flt3, c-Kit, PDGFR	AML Solid tumors
PD 0325901	Pfizer	MEK	Solid tumors
BAY 43–9006	Bayer/Onyx	BRAF, VEGFR1–3	Malignant melanoma Renal cell carcinoma

AML: Acute myeloid leukemia; CML: Chronic myeloid leukemia; CMML: Chronic myelomonocytic leukemia; EGFR: Epidermal growth factor receptor; GIST: Gastrointestinal stromal tumor; HES: Hypereosinophilic syndrome; NSCLC: Non-small cell lung cancer; PDGFR: Platelet-derived growth factor receptor; VEGFR: Vacular endothelial growth factor receptor.

patient-derived imatinib-resistance mutations. Interestingly, a large number of mutations were found outside the kinase domain. As most studies of patient-derived blasts have been restricted to this domain, it is presently unclear whether these mutations represent experimental artifacts or would be compatible with the biologic ability of Bcr-Abl to drive CML. These studies also indicate that it should be possible to predict which mutated clones will arise in response to treatment with a particular drug, although the large number of identified mutations implies that it will be very difficult to identify a single therapeutic agent that could overcome all cases of resistance. Currently, attention is being focused on design of inhibitors that can overcome individual or a series of resistance mutations. More potent imatinib-like inhibitors could be used against those mutants that affect the conformation adopted by the kinase as it binds to the drug or those that occur outside the kinase domain. Similarly, such inhibitors should be effective against resistance due to amplification or overexpression of Bcr-Abl. These inhibitors are not expected to be active against mutants that alter residues that directly interact with imatinib, such as the Thr315Ile mutant. Instead, inhibitors that directly target the active conformation may be useful, and several examples of this are known: PD-180970 has been shown to inhibit imatinib-resistant mutants of Bcr-Abl [50], and the Thr674Ile imatinib-resistant mutant of FipIL1-PDGFR was shown to be more sensitive to the staurosporine analog PKC412 than wild type Fip1L1-PDGFR [51]. Recently, BMS-354825, a dual Src/Abl inhibitor which is a 100-fold more potent inhibitor of Abl than imatinib, has been demonstrated to inhibit many tumor-derived, imatinib-resistant Abl mutants in preclinical mouse studies (Thr315Ile being a notable exception) [52]. Although the lack of selectivity of such agents may restrict their use in the clinic, a promising clinical response has recently been reported upon treatment of imatinib-resistant GIST with SU011248, an orally active inhibitor that targets several kinases, including PDGFR, Flt-3, c-Kit and VEGFR. More recent publications have demonstrated that the molecular mechanisms underlying imatinib resistance in GIST are similar to those observed in CML [53], suggesting that SU011248 acts via inhibition of imatinib-resistant c-Kit and PDGFR point mutants. However, additional effects via its action on compensatory pathways that are deregulated in the resistant tumors cannot be discounted [54].

Targeting parallel or downstream signaling pathways may represent a general approach by which resistance may be overcome. Bcr-Abl transformation results in activation of mitogen-activated protein kinase (MAPK), Akt and c-Src pathways. Inhibitors for these pathways are either in preclinical or clinical development and there is already evidence of synergistic or additive effects in combination with imatinib in model systems [55–57]. Increased understanding of the factors that modulate the effectiveness of combinational therapy, together with early identification and characterization of emerging resistance, are imperative steps in the design of more effective therapy.

EGFR inhibitors

The EGFR family of receptors consists of four structurally related transmembrane RTKs [58–61]. Ligand binding induces receptor hetero- and homodimerization and activation of the intrinsic tyrosine kinase domain. ErbB2/HER-2, that lacks any known ligand, is the preferred partner for the other receptors, however, the dimerization partner is also ligand and cell type dependent. Kinase activation results in initiation of downstream signaling cascades such as the PI3K/protein kinase B and MAPK pathways [60]. Overexpression of EGFR family members is frequently observed in solid tumors [62], whereas receptor mutations are less frequent, with the notable exception of brain tumors where both amplification and activating extracellular domain mutations have been reported. Mutation or high expression levels of EGFR and HER-2 are correlated with poor prognosis and more aggressive disease, and has led to intense efforts in identifying and developing therapeutic entities directed against these kinases [63,64].

Amongst these agents, the most advanced include small-molecule inhibitors (e.g., gefitinib and erlotinib) and therapeutic antibodies (e.g., trastuzumab, pertuzumab and cetuximab). Trastuzumab, a humanized immunoglobulin G1 antibody directed against HER-2, was the first FDA-approved monoclonal antibody directed against solid tumors. In a pivotal Phase III trial of patients with HER-2-positive metastatic breast cancer, combination of trastuzumab with chemotherapy significantly increased response, median duration of response and overall survival when compared with chemotherapy alone [65,66]. Trastuzumab has also demonstrated activity as a first-line single-agent therapy of patients with HER-2/3-overexpressing metastatic breast carcinoma [10]. Adverse effects have been documented, including cardiac dysfunction and narrowing of blood vessels, particularly when trastuzumab has been used in combination with anthracyclins [67].

Trastuzumab and pertuzumab bind to different epitopes on the extracellular domain of HER-2 [68]. Although both antibodies induce ADCC, pertuzumab fails to inhibit shedding of the intracellular domain of the receptor [69]. Interestingly, unlike trastuzumab, pertuzumab is effective in inhibiting HER-2 signaling in cell lines that do not overexpress the receptor [70]. This effect does not appear to be due to ADCC effects as the pertuzumab Fab domain is an equally effective inhibitor. Instead, pertuzumab binding blocks HER-2 signaling by preventing heterodimerization with coreceptors [68]. As expected, pertuzumab is effective both in the context of HER-2 overexpression, as well as in those tumors where HER-2 is transactivated via ligand-mediated activation of coreceptors (e.g., HER-3 and EGFR) [71,72]. Pertuzumab has shown an acceptable safety profile and has progressed to Phase II clinical trials [68].

Cetuximab is a humanized antibody that competes with ligand to bind the EGFR [73]. It has been reported that antibody binding results in receptor internalization without kinase activation. The consequent downregulation of signaling is associated with increased apoptosis, however, enhanced ADCC has also be reported [74,75]. The results from a Phase II clinical study led to an approval by the FDA for the use of cetuximab in combination with irinotecan in the treatment of patients with EGFR-expressing, metastatic colorectal cancer who are refractory or intolerant to chemotherapy. Cetuximab has also shown activity in patients with advanced pancreatic cancer upon combination with gemcitabine, a deoxycytidine analog, that represents the standard first-line chemotherapy for pancreatic cancer. Adverse effects observed upon administration of cetuximab included infusion symptoms as well as frequent (75%) occurrence of an acne-like rash, a common reaction to treatment with EGFR inhibitors. Rash could be used as a predictor of the survival of patients, the patients with the most intense rash deriving the greatest benefit from treatment [76].

Both gefitinib (EGFR inhibitor) and erlotinib (EGFR/HER-2 inhibitor) displayed activity against a wide spectrum of cell lines, as well as inhibiting growth and angiogenesis of tumor xenografts [77–80]. Activity hints were observed in Phase I clinical trials in patients with tumors expected to express high EGFR levels, including colorectal cancer, non-small cell lung cancer (NSCLC) and head and neck squamous cell carcinoma (HNSCC) [81,83]. Similar objective response rates, approximately 10%, were observed in single-agent Phase II trials of both gefitinib and erlotinib in chemotherapy-refractory NSCLC patients. Although response rates were low, they were frequently durable and rapid in onset. This, in combination with an acceptable safety profile, led to the approval of gefitinib for treatment of NSCLC patients who had previously undergone chemotherapy, first in Japan, where a particularly high (19%) response rate was observed, and subsequently by the US and Australian regulatory authorities [84]. Unfortunately, Phase III trials in which gefitinib or erlotinib was combined with chemotherapy failed to demonstrate any improvement in response rate, progression-free or overall survival in NSCLC patients when compared with treatment with chemotherapy alone [80,85].

Issues in the clinic: patient selection

A large number of studies have been carried out to identify the reasons underlying the low patient response in trials with EGFR antagonistic agents. Post-treatment analysis of patient samples failed to demonstrate a clear correlation between the expression of EGFR and response to these drugs, thereby underlining a central problem in design of clinical trials – a lack of clear definition of which patients would benefit from kinase inhibitor therapy. In a similar manner, although a clear correlation existed between expression levels of HER-2 and response to trastuzumab monotherapy, less than a third of patients with receptor-positive malignancy benefited from treatment. The advent of validated antibodies that specifically recognize phosphorylated, and therefore activated, receptors has provided a potential explanation for this observation. Wide, tumor type-dependent variations in the percentage of tumors that contained phosphorylated receptor were observed in a post-treatment study of HER-2-overexpressing breast carcinomas [86,87]. Progression-free survival of patients with tumors containing phosphorylated HER-2 was found to be significantly longer after treatment with trastuzumab, when compared with patients with tumors lacking phosphoreceptor. Although these studies highlight the potential use of such an approach to identify tumors containing an activated kinase, it is apparent that intact transmission of the receptor signal to intracellular targets is also required. Resistance to gefitinib correlated with a failure to inhibit MAPK and Akt activation, despite blocking EGFR phosphorylation [88]. Further studies indicated that reconstitution of the tumor suppressor PTEN, a negative regulator of the PI3K/Akt pathway, restored sensitivity to EGFR inhibition [89]. Greater characterization of the activation state of an overexpressed kinase, its downstream signaling intermediates, as well as other signaling pathways that impinge on its action, are therefore imperative in defining a population of patients that would respond to an inhibitor. In the absence of a clear understanding of these factors, subpopulations of patients that do benefit from treatment risk being overlooked.

Importantly, two recent publications have provided the first instance of biomarkers that may be used to define tumor sensitivity to gefitinib [8,9]. Sequencing the EGFR tyrosine kinase domain identified a series of heterozygous point mutations that correlated with responsiveness of tumors to gefitinib. The presence of mutations mirrored the documented sensitivity to gefitinib, being more common in Japan, in adenocarcinomas, in women and in nonsmoking patients. Mutated EGFR displayed prolonged ligand-stimulated activation and increased sensitivity to gefitinib.

Although these studies provide hope that it will be possible to define a population of patients likely to be responsive to treatment with gefitinib, they raise additional questions. Does the absence of EGFR mutations in a responsive patient mean that mutations of additional pathways also render cancers sensitive to the effects of the drug? Does the different responsiveness of Japanese and US patients reflect different etiology of the disease due to racial, or as has been suggested, cultural (dietary) differences? Examination of more samples, including those from a recently reported trial of erlotinib, is essential to strengthen the statistical significance of these studies and to provide some answers to these questions.

VEGFR

VEGF and its cognate receptors, VEGFRs, play an essential role in angiogenesis, the process by which capillaries sprout from established blood vessels [90]. Simple diffusion of nutrients and oxygen becomes insufficient as tumors grow beyond a certain size, necessitating the *de novo* establishment of a blood supply [91,92]. VEGF expression is elevated in hypoxic and hypoglycemic conditions, upon expression of oncogenes, such as Ras, or due to inactivation of tumor suppressor genes, such as p53 or the von Hippel-Lindau gene [93]. Elevated levels of VEGF have been associated with poorer prognosis and increased chance of metastasis in a number of tumors [93]. In preclinical models, blockage of VEGF signaling with inhibitory antibodies inhibited the growth of tumor xenografts and reduced the number of metastases [94]. The proven role for VEGF in the regulation of vessel permeability suggests that its inhibition would also decrease the elevated interstitial pressure observed within tumors. Decreased interstitial tumor pressure would enhance delivery and therefore the effectiveness of coadministered chemotherapeutic agents, as has been observed in xenograft models [95].

A number of approaches have been adopted in targeting VEGF signaling. These include the use of therapeutic antibodies such as bevacizumab, monoclonal antibodies such as IMC-1C11 [96], small-molecular-weight inhibitors such as SU5416, SU-6668, SU11248 [97–99] and vatalanib (PTK787/ZK222584) [100], as well as angiozyme, a stabilized ribozyme that targets the premessenger RNA of VEGFR1 [101]. The most clinically advanced of these is bevacizumab, which binds to VEGF, inhibiting interaction with its cognate receptors. In a Phase II trial on patients with metastatic clear cell kidney carcinoma, no alteration in primary tumor growth or overall patient survival was observed, however, a significant difference in time to progression was observed in those patients receiving bevacizumab compared with placebo [102,103]. However, most trials have focused on the combination of bevacizumab with chemotherapeutic agents. Combination with capecitabine, a fluoropyrimidine carbamate that inhibits thymidylate synthase, or vinorelbine, a vinca alkaloid, resulted in increased objective response rates in patients with metastatic breast cancer. Increased response rates and time to disease progression were also observed when NSCLC patients were treated with a combination of high-dose bevacizumab and carboplatin/paclitaxel [15]. Bevacizumab has recently been approved by the FDA as a first-line treatment, in combination with 5-fluorouracil/leucovorin/irinotecan therapy, for treatment of patients with metastatic colorectal cancer following results of Phase III trials where combination of bevacizumab with chemotherapy significantly increased overall survival, objective response rate and progression-free survival [104–106]. In these studies, no surrogate markers of activity were reported, and it therefore remains to be clarified whether the effects of bevacizumab are due to direct inhibition of neovascularization or modulation of vascular permeability.

Issues in the clinic: surrogate end points

Traditionally, anticancer drugs have been clinically evaluated in three phases. Phase I trials focus on drug safety and identification of an optimal dose and schedule for subsequent trials. Successive small patient groups are given incrementally higher concentrations of drug until a maximum tolerated dose (MTD) can be identified. Phase II trials are designed to evaluate the effectiveness of the MTD of the drug by measuring clinical responses (i.e., decrease in tumor size following treatment). The drug then enters Phase III trials where its efficacy, in terms of patient survival, is compared with that of the currently accepted standard treatment for that tumor type.

In contrast to cytotoxic agents, kinase inhibitors may have little/no toxicity and stable disease, rather than reduction in tumor size, should be considered a successful therapeutic outcome. Although most kinase inhibitors have, to date, been assessed using a classic trial design, these peculiarities make renewal and adaptation of clinical trials methodology essential. To reflect the proposed mechanism of action of the agent being studied, it is more appropriate to consider the use of biologic end points and surrogate markers. Instead of a MTD, Phase I studies should be designed to define the dose of a drug that provides maximal or sufficient target inhibition, that is the maximal biologically effective dose (MBD). Currently, however, the technology required to assess a MBD for most kinase inhibitors in clinical tissue is lacking. Therefore, most kinase inhibitor programs are accompanied by a parallel effort in translational research to develop robust, predictive and well-controlled biomarker assays. Approaches include generation and validation of phosphorylation-specific antibodies to detect modification of kinase or substrate in clinical tissue by immunochemistry, as well as the use of proteomics and expression profiling to identify treatment/response-dependent signatures.

Imaging studies to assess changes in tumor metabolism or vascular architecture have also been developed as alternative methods to assay compound activity. Dynamic, contrast-enhanced molecular resonance imaging (DCE-MRI) studies have been utilized to demonstrate that treatment with anti-VEGF agents (bevacizumab and PTK787) induces alterations in vascular structure and permeability [107,108]. Analysis of ^{18}F-fluorodeoxyglucose uptake by positron emission tomography following treatment of GIST patients with imatinib correlates with the activity of this drug in this highly metabolically active tumor. Relapse in patients undergoing treatment with the drug is accompanied by the reappearance of areas of metabolically active tumor [109].

During Phase I trials with imatinib, a clear correlation between clinical efficacy and inhibition of Bcr-Abl kinase activity in circulating leukemic cells was observed. However, as many kinase inhibitors are directed against solid tumors, from which it is inconvenient and frequently impossible to obtain biopsies to directly assess target inhibition, surrogate markers of drug efficacy are of particular importance. Although serendipitous clinical observations, such as rash (EGFR inhibitors) or alteration in hair color (c-Kit inhibitors), are of use in predicting treatment outcome and confirming patient compliance, assays measuring target kinase activity in a surrogate tissue are being developed for most kinase inhibitors entering trials. Surrogate biomarkers can also be used to provide information about the magnitude and durability of response to an inhibitor (i.e., how much kinase activity needs to be inhibited for how long to be clinically effective). CI-1040/PD 184352 is a non-ATP competitive MEK inhibitor that displayed activity against a range of tumor xenografts [110]. The phosphorylation of a MEK target, MAPK, in patient blood and tumor specimens, was used as a biomarker to demonstrate an acceptable safety profile at doses sufficient to inhibit the kinase. A failure to maintain target inhibition was probably responsible for the reported lack of activity of CI-1040 as a single agent in Phase II trials. Subsequently, PD 0325901, a more potent and soluble second-generation analog of CI-1040, has been introduced into Phase I clinical trials.

Clearly, the use of surrogate markers requires sufficient data on how reproducible, as well as how representative, they are in defining the threshold of the biologic response in the tumor tissue. The rapamycin derivatives, CCI-779 and RAD001, are currently in clinical trials with patients with advanced solid cancers. The target of both drugs, TOR, regulates translation of specific mRNA species with central roles in control of cell size and proliferation. Both drugs display antiproliferative activity as single agents in a range of preclinical cellular and xenograft systems [21]. CCI-779, the clinically more advanced compound, was evaluated using a traditional dose-escalation approach in Phase I trials. Mild-to-severe toxicities, including evidence of hepatic impairment, vomiting and thrombocytopenia, were observed in heavily pretreated patients. Notably, in a second study, based on a weekly rather than biweekly schedule, no clear correlation between dose and adverse events was observed [111]. Partial response was observed in four patients with diverse tumor types, including NSCLC and renal cell carcinoma. In a Phase II trial with renal cell carcinoma patients, based on these results, objective response in 7% of patients and stable disease in 40% has been reported [112]. In contrast, during preclinical studies with Rad001, a relationship between unbound compound concentration and inhibition of a TOR substrate, p70S6K1, in peripheral blood mononuclear cells (PBMCs) and implanted tumor was established [113]. In Phase I trials, groups of patients received a single concentration of Rad001, calculated to achieve plasma concentrations sufficient to inhibit TOR [114,115]. Assessment of p70S6K1 activity in patient PBMCs was used as a marker of compound activity. In corroboration of these results, inhibition of p70S6K1 activity in PBMCs was found to significantly correlate with time to progression in post-treatment studies with CCI-779 [116]. Although further studies that correlate kinase inhibition in patient-derived tumor and PBMCs are required, these reports provide an example of how assessment of a surrogate marker may be utilized to provide a relevant pharmacodynamic readout of the efficacy of a kinase inhibitor.

Information provided by biomarkers and surrogate markers should also be incorporated into the design of Phase II clinical trials. Previously, large sample sizes were required due to the necessity to treat randomized patient populations with histologically defined tumors with a range of drug doses to assess both activity and toxicity issues. In contrast, once the MBD of a kinase inhibitor has been established, these trials should be less cumbersome, treating smaller numbers of patients with cancers known/likely to be dependent on the kinase targeted with doses close to the MBD. The end points of these trials should also be modified; assessment of shrinkage in tumor size is no longer appropriate to agents that will be largely cytostatic. Instead, if the new drug is active, it will modify the expected natural history of the disease. This can be measured, within each patient, by comparing time to progression on the new drug with time to progression registered with the previous first-line treatment. However, the heterogeneity in growth of many solid cancers will still necessitate recruitment of sufficient numbers of patients to distinguish between those tumors responding to the drug and those that naturally grow slowly. As a further refinement, Ratain and coworkers have proposed the randomized discontinuation design [117]. In this trial design, all patients are initially treated with the agent then patients with stable disease are randomized into placebo and continuing therapy groups. By initially selecting a more homogenous population, fewer patients should be required to demonstrate the activity of a drug. BAY 43-9006 (Sorafenib), a Raf inhibitor (IC_{50} <10 nM) that also displays activity against VEGFR1–3, c-Kit and PDGR (IC_{50} 10–160 nM), has shown activity in Phase I studies of a cohort of 163 patients with a range of solid tumors [118]. Seven patients with renal cell carcinoma were treated with BAY 43-9006. Out of these patients, one displayed a partial response and five reached stable disease. Although the target kinase in these tumors remains unresolved, renal cell carcinoma rarely contains mutations in BRAF, a Phase II trial incorporating the randomized discontinuation design was initiated. Preliminary data published from the trial indicate that after treatment with BAY 43-9006, 30% of patients had stable disease, while 40% responded (defined as >25% reduction in tumor volume as assessed by computed tomography or magnetic resonance imaging) [119]. Patients with stable disease were then randomized according to the trial design. Those patients that progressed while receiving placebo were rechallenged with the drug, whereas those progressing with the drug were taken off study. Although the final results of this trial are awaited, the preliminary findings using this trial design have prompted interest in the use of BAY 43-9006 for the treatment of renal cell carcinoma, and have led to a further Phase III study which is due to complete accrual in 2005.

Summary & conclusions

Advances have been made both in identifying the role of kinases in malignancies and characterizing inhibitors that can block their activity in a therapeutically relevant manner. The clinical efficacy of the compounds described herein demonstrates the utility and potential of kinase inhibitors, but also delineates a series of ground rules necessary for success. Central to these is a thorough molecular characterization of the role a kinase plays in the pathophysiology of a particular cancer. This is obviously a great challenge and can only be achieved by analysis of statistically relevant numbers of clinically defined samples. Experience with imatinib, and the recently reported studies of tumors responsive to gefitinib, suggest that activating gain-of-function mutations are more predictive than overexpression in defining a fundamental role for a kinase in cancer development. However, it is also apparent that the cellular context plays an important role, as exemplified by the higher probability of relapse to imatinib treatment in blast crisis patients with ALL rather than CML [120].

The emergence of resistance may be an inevitable consequence of any clinically effective kinase inhibitor. Specific point mutations that render kinase activity insensitive to the presence of inhibitor may be more likely to occur upon treatment with conformation-specific drugs, such as imatinib. Biochemical and structural studies have elucidated how these mutations overcome the action of the inhibitor and suggest methods of overcoming resistance. One approach, namely the treatment with a less specific inhibitor that interacts with all conformations of the kinase, has been attempted with some success in GIST patients who relapsed on treatment with imatinib. However, it should be noted that the inherent genetic instability of advanced stage tumors is likely to result in the appearance of other mechanisms of resistance to these agents. Alternative approaches such as targeting kinases expressed on nontransformed cells such as VEGFR, Tie-2 or PDGFR on endothelial cells or fibroblasts, where the emergence of resistance would be less likely, or targeting multiple mutant kinases by inhibiting the action of molecular chaperones such as heat shock protein 90, may provide clinical benefit in these circumstances.

Expert opinion

Personalized medicine & targeted therapy

The past 5 years of clinical experience with kinase inhibitors has underscored the problems associated with employing targeted therapy against a molecularly heterogeneous disease. Differences between histologically similar cancer types has necessitated accrual of larger patient populations and led to the risk of clinical trials becoming underpowered, since beneficial effects on small percentages of patients are masked by the lack of response in the majority of patients. Furthermore, heterogeneity within a single tumor can result in outgrowth of subclones that do not possess the altered kinase activity, resulting in only partial response to treatment with an inhibitor. Molecular analysis of tumor tissues, taken before and after treatment, is beginning to permit characterization of those factors that identify drug sensitivity. Inevitably, these studies will also result in subfractionation of patient populations reducing the numbers that would benefit from administration of a particular kinase inhibitor. On the other hand, these studies should also help to identify aberrant activation of other pathways that represent potential targets. The low toxicity inherent in the premise of targeted therapy should permit the combination of inhibitors of different pathways allowing the development of treatment protocols tailored for individual patients. Targeting multiple aspects of the pathophysiology of a disease has already met with success in HIV therapy and examples of the beneficial effects of combinations of kinase inhibitors have already been reported in preclinical studies [121]. Personalized treatment strategies should provide the maximum benefit for the patient in terms of progression-free survival and improvement in quality of life, but will inevitably be associated with huge increases in the cost of patient healthcare.

Five-year view

If the last 10 years has seen the validation of kinase inhibitors as weapons in the war on cancer, it can be anticipated that the next 5 years will see the rationalization of their use in those situations where they will be predicted to have the maximal beneficial effect. Development of robust and validated diagnostic techniques to detect kinase activation in biologically relevant tissue, together with more appropriate design of clinical trials, will allow more effective evaluation of the clinical activity of new molecular entities. Increases in the armament of validated kinase inhibitors will be accompanied by design of trials utilizing the combination of agents, based on a clear mechanistic understanding of their actions in an appropriate cancer tissue. The increased cost of such an approach would be predicted to be accompanied by significant improvement in the clinical effectiveness of cancer treatment.

Key issues

- Sufficiently specific inhibitors can be developed with an acceptable safety profile.
- The development of preclinical models/assays that accurately reflect the role of kinases in tumor development is needed.
- Appropriate patient selection is essential to identify patients who would receive maximum benefit from treatment with a kinase inhibitor.
- Robust, accurate and sensitive biomarkers/surrogate markers need to be incorporated into clinical trials.
- Heterogeneity of the tumor microenvironment will require patient-specific drug combinations as well as continual monitoring of the tumor in terms of development of resistance and outgrowth of different clones.

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