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Validating cancer drug targets through chemical genetics

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Abstract

Targeted therapies for cancer promise to revolutionize treatment by specifically inactivating pathways needed for the growth of tumor cells. The most prominent example of such therapy is imatinib (Gleevec), which targets the Bcr-Abl kinase and provides an effective low-toxicity treatment for chronic myelogenous leukemia. This success has spawned myriad efforts to develop similarly targeted drugs for other cancers. Unfortunately, the high expectations of these efforts have not yet been realized, likely due to the genetic diversity among and within tumors, as well as the complex and largely unpredictable interactions of drug-like compounds with innumerable targets that affect cellular and organismal metabolism. While improvements in sequencing technologies are beginning to address the first problem, solving the second problem requires methods for linking specific features of the cancer genome to their optimally targeted therapies. One approach, referred to as chemical genetics, accomplishes this by genetic control of chemical susceptibility. Chemical genetics is a crucial tool for the rational development of cancer drugs.

1. Introduction

The essence of chemical genetics lies in defining drug selectivity—resistance or sensitivity—through genetic alteration of the target(s) of interest. Such alteration can be achieved by natural selection or by directed manipulation in a genetically tractable model system or organism. Because chemicals can affect complex biologic systems via many potential targets, genetics allows their effects to be linked to individual targets.

Chemical genetics can be carried out using either *resistant* or *sensitive* mutations (Figure 1). The former class of mutations renders the target enzyme resistant to chemical inhibition (Fig. 1A). As shown below, this can reveal which enzymes are important mediators of a given chemical's *in vivo* effects, and thus can help to disentangle some of the complexity associated with drug-like molecules that affect multiple targets in the cell. Conversely, sensitive mutations (Fig. 1B) deliberately enlarge an enzyme's active site to accommodate bulky non-natural ligands. In most cases, sensitized mutants are then substituted for the endogenous version of the enzyme in cells or whole organisms. Both types of chemical genetics are beneficial: whereas resistant mutations can identify the targets of currently available drugs, sensitive mutations allow one to interrogate gene function independently of the existing pharmacopoeia.

How does chemical genetics inform cancer drug discovery? First, chemical genetics can establish whether inhibition of a particular target is beneficial *in vivo*, and thus worth the considerable time, effort, and cost of drug development. Second, chemical genetics can

reveal what an effective drug's physiologically relevant targets are, regardless of its apparent selectivity (or lack thereof) *in vitro*. Because of these effects, chemical genetics is increasingly important for development of rational therapeutics.

2. Current paradigms in cancer drug discovery

2.1 Protein kinases are excellent drug targets

Although chemical genetics is valuable for all classes of targeted therapies, it is especially relevant for protein kinases. The human genome encodes over 500 protein kinases ('the kinome'), representing the largest class of enzymes [1]. The catalytic domains of kinases are structurally conserved and readily identifiable through sequence homology. Protein kinases have the ability to bind ATP and catalyze the transfer of the gamma phosphate to a hydroxyl group of a substrate protein on a tyrosine, serine, or threonine residue. Despite the high sequence homology, protein kinases have selectivity for specific protein substrates conferred by localization, timing of activation and sequence specificity encoded in the substrate-binding domain. For example, the cyclin-dependent kinases (CDKs) preferentially phosphorylate serines or threonines adjacent to proline, a feature which aids in the identification of their *in vivo* substrates.

Kinases represent outstanding targets for drug therapy because the ATP-binding domain has evolved for high-affinity binding to this small molecule. Thus, this site is amenable to high-affinity binding by drugs which can competitively inhibit ATP binding and hence catalytic activity. However, these ATP sites are also conserved across all members of the human kinome so chemical inhibitors frequently affect many. Nevertheless, due to small differences in the ATP-binding domain, some degree of selectivity can be achieved and some inhibitors have high affinity to a small number of kinases.

2.2 Protein kinases and cancer

A common mechanism of oncogenesis involves the constitutive activation of protein kinases via translocation, point mutation, or locus amplification (Table 1). Activation of oncogenes is an important early event in the transformation of normal cells to malignant cells. Nevertheless, it has not always been clear if sustained activity is required throughout the tumor's evolutionary lifespan. One attractive hypothesis (sometimes referred to as the 'oncogene addiction' model) states that established tumors indeed require persistent activation and interrupting the oncogenic signaling of established tumors can be therapeutic. Recent evidence for oncogene addiction has emerged from both murine models [2] and clinical experience with CML, gastrointestinal stromal tumors (GIST), and EGFR-mutant lung cancer, where specific inhibition of oncogenic kinases results in tumor regression [3]. Non-oncogenic protein kinases may also be good drug targets if inhibition leads to selective cell death of cancer cells, often termed synthetic lethal.

Based on their function, most protein kinases of interest can be divided into distinct classes: receptor tyrosine kinases (RTKs), mitogenic signaling kinases, and cell cycle kinases.

2.2.1 Receptor tyrosine kinases—RTKs represent an important group of oncogenic protein kinases and molecular targets for cancer therapy (Figure 2). These include the epidermal growth factor receptor (EGFR/ErbB2) family of kinases, platelet-derived growth factor receptor kinases (PDGFR), and insulin-like growth factor kinases (IGF-1, IGF-2). RTKs typically are activated by binding extracellular ligand. This mediates transmembrane signaling through dimerization and autophosphorylation on cytoplasm-facing tyrosine residues. These residues are docking sites for downstream effectors that bear phosphoselective protein-binding domains. Constitutive activation of certain RTKs is

oncogenic, such as occurs with EGFR mutation in lung adenocarcinomas [2,4], ErbB2 (Her2) amplification in breast cancers[5,6], and PDGFR β in myeloproliferative syndromes [7]. Thus, these RTKs involving kinases are important targets for approved treatments for cancer (e.g. by the United States Food and Drug Administration, FDA). These drugs fall into two classes: (1) antibodies targeting extracellular domains, which elicit antibody-mediated cell cytotoxicity and other mechanisms of anticancer effects [8], and (2) small molecule inhibitors of kinase domains. The latter category includes FDA-approved inhibitors of EGFR for lung cancer (erlotinib) and EGFR/Her2 in breast cancer (lapatinib).

Included among the RTKs are the vascular endothelial growth factor receptors (VEGFR). These receptors promote growth of blood vessels that are required to nourish growing tumors (angiogenesis). An antibody against VEGF, the receptor ligand (bevacizumab or Avastin), is approved in the United States for treatment of cancers of the lung, breast, and colon. In theory, drugs can inhibit VEGFR in addition to other kinase targets and can lead to regression of blood vessels and tumor—two multitargeted kinase inhibitors (sunitinib and sorafenib) are thought to be effective kidney cancer drugs via antiangiogenic effects. It remains unclear whether the therapeutic effects are due to direct inhibition of VEGFR or other targets and whether the therapeutic effects are elicited in endothelial cells or in tumor cells.

2.2.2. Kinases involved in mitogenic signaling—Mitogenic signaling cascades represent a second class of targets important for cancer therapy (Figure 2). These cascades typically transduce signals from RTKs or other stimuli to regulate gene transcription and cell cycle. These signals promote survival, proliferation, and invasion of cancer cells. Mitogenic signaling cascades relevant to cancer include the phosphoinositide-3-kinase (PI3K, a lipid kinase) pathway and the RAS pathway. Constitutive activation of these pathways confers an oncogenic stimulus. For example, within the PI3K pathway, activating mutations have been observed in PIK3CA and Akt1 [9], and in the RAS pathway, mutations are frequently found in KRAS and BRAF. These have been validated as targets of cancer therapy; for example, temsirolimus blocks mTOR and is approved for treating kidney cancer. Preliminary reports of phase I trials suggest that some BRAF inhibitors are highly effective for treating melanoma [10]. Numerous inhibitors of PIK3CA, Akt, BRAF, MEK and mTOR are in clinical development.

2.2.3. Cell cycle kinases—Another family of kinases of interest for cancer therapy includes the cell cycle kinases (Table 2). These include CDKs, Aurora kinases, Polo-like kinases (Plks), and other regulators of the cell cycle which are essential for cell proliferation and preservation of genome stability. By targeting these processes, inhibitors of these kinases have at least theoretical potential to suppress cancer cell proliferation, but at the same time, may be toxic to non-transformed tissues with high proliferative indices (e.g., gut and bone marrow). However, certain characteristics of cancer cells, such as chromosome instability, polyploidy, or activation of specific oncogenic pathways can make them more susceptible to drug inhibition than proliferative non-transformed tissues.

One example is polo-like kinase 1 (Plk1), which plays key roles in orchestrating mitosis of cancer cells and non-cancer cells alike. However, cancer cells are more susceptible than non-transformed cells to Plk1 knockdown or inhibition [11,12]. Two unbiased screens have recently elucidated the mechanism of this observation in tumors with KRAS activation and p53 loss. These represent some of the most frequent genetic alterations observed in cancer, but are classically regarded as ‘undruggable’ targets. In the first study, the Elledge group performed an siRNA screen to identify potential drug targets in KRAS mutant cells [13]. To do this, they identified siRNAs that lead to cytotoxicity in mutant cells but not in isogenic cells with wildtype KRAS, then verified results with chemical inhibitors. This study

revealed that Plk1 inhibitors were highly effective in blocking proliferation of KRAS mutant cells, but less toxic to cells with wildtype KRAS. Similarly, the Vogelstein group found that inhibition of Plk1 is effective in cells lacking p53[14]. Thus, these studies suggest that Plk1 inhibitors are likely to be highly effective with a large therapeutic window via a tumor-specific 'synthetic-lethal' interaction that could be productively exploited for cancer therapy[13,14].

2.2.4. The 'dark matter' of the kinome—The human genome encodes over 500 protein kinases but the functions of most remain obscure. A recent survey shows a disproportionate focus on a small number of the protein kinases among academic studies. In contrast, a large number of largely unstudied kinases are expected to make good targets, as evidenced from RNAi screens or oncogenic driver mutations [15]. Over 50% of the human kinome remains largely uncharacterized and more than 100 members have completely unknown function. Yet, these may represent crucial mediators of on- and off-target effects of existing and future kinase-targeted drugs. Elucidating these kinase functions is therefore crucial for developing targeted cancer drugs. When the functional significance of the kinome is catalogued, drugs can be specifically developed to avoid hitting kinases that mediate activity. Conversely, drug effects against kinases with relatively little function in the adult organism may not need investigation.

3. How specific are current kinase-targeted therapies?

To date, eight inhibitors of protein kinases are approved by the FDA for treating cancer (Table 3). Although each was developed with a drug target in mind, off-target effects of these drugs have assumed significant importance in their clinical use. Table 3 lists the number of kinases that is affected by each drug in biochemical assays of binding [16] or activity (Selectscreen®, Invitrogen). Conventionally, drug targets have been identified through the isolation of stable ligand-enzyme interactions or screening in activity-based assays across large panels of recombinant kinases [16]. Both methods have exposed variable promiscuity among ostensibly selective protein kinase inhibitors. Among the FDA approved agents, sunitinib (kidney cancer) has the greatest number of protein kinase targets, whereas lapatinib (Her2+ breast cancer) appears to be highly selective for inhibition of EGFR1/Her2 (Table 3). While useful, such *in vitro* assays often deviate significantly from *in vivo* reality, due to the lability of the drug-enzyme complex and/or omission of physiologically relevant regulators and substrates. Thus, validating drug selectivity in living cells and organisms remains a challenging but essential step in drug development. Fortunately, chemical genetics provides several strategies for achieving this objective.

For kinase inhibitors, the selectivity, or lack thereof, has affected clinical development, as illustrated by imatinib (Gleevec) and sorafenib. Imatinib was developed as a selective inhibitor of Abl kinase for CML, and has provided a highly effective (but not curative) alternative to bone marrow transplant [17]. However, imatinib was subsequently found to be a high affinity inhibitor of c-Kit and PDGFR α/β [18]. This made it an attractive drug for treating GI stromal tumors (GIST) which harbor activating mutations in c-Kit, and myeloproliferative disorders mediated by PDGFR β . Thus, other targets were useful in expanding use of the drug to other indications. This allowed rational use of a drug with limited selectivity.

Sorafenib is another case study in the importance of off-target functions. Sorafenib (BAY 43-9006) was developed as a potent inhibitor of Raf kinase, selected as a drugable target mediating oncogenic signaling from KRAS [19]. Combinatorial libraries were screened with Raf kinase assay. Active compounds were secondarily analyzed by detecting a Raf-mediated phosphorylation and ability to block KRAS mutant cancer cells from proliferation and

growth in soft agar. Next, hits were counterscreened for blocking downstream elements of the KRAS-Raf pathway. Finally preclinical data supported use of sorafenib for KRAS+ tumors and this was moved into clinical development. At the same time, effect of this drug on other protein kinases was analyzed in biochemical assays, defining other targets[20]. Ultimately, the focus of clinical development included kidney and liver cancer, in which no effective chemotherapies were available. Sorafenib was approved in the U.S. for unselected patients with kidney and liver cancer, although KRAS mutations are rare in these diseases (~1% of kidney cancers and 4% of liver cancers according to COSMIC database [21]). At present, the mechanism of sorafenib's action against these malignancies remains obscure. Its efficacy has been variously attributed to effects on VEGFR2/3, PDGFR β , Flt-3, and c-Kit, although *in vitro* assays have defined up to 32 protein kinase targets.

These case studies demonstrate that development of 'targeted drugs' can proceed rationally or empirically. Drugs with moderate selectivity, such as imatinib can be rationally used for a number of targets, but when selectivity is poor, clinical development is empiric. Identification and validation of drug targets allows a rational approach to drug development. Below we discuss how such measures can be established through chemical genetics.

3. Validating drug targets through acquired resistance

One way of linking a drug with its true physiologic targets is to exploit natural selection. Specifically, spontaneous mutations that prevent the drug from binding to its bona fide target should also reverse the effects of the drug on intact cells, tissues, and organisms. This principle has been termed 'forward chemical genetics,' by analogy to classical genetic screens [22]. Consistent with this logic, CML patients who relapse during imatinib therapy frequently harbor mutations that hinder the Bcr-Abl kinase's interaction with this compound, often due to mutational narrowing of the enzyme's catalytic cleft [17]. Although imatinib is able to affect other kinases such as c-Kit and PDGFR, the rescue mutations in Abl kinase clearly define this as a key target required for imatinib function. (Although it does not formally rule out the possibility that simultaneous inhibition of c-Kit, for example, is required for the therapeutic effect[23]). Thus, future drug development in CML can be directed at more selective inhibitors to reduce toxicity or to overcome mechanisms of resistance. Similarly, natural selection of cancer has demonstrated EGFR as the key target of gefitinib and erlotinib [24]. These findings validate Abl and EGFR as targets of cancer therapy.

In addition to linking drugs with their targets, the isolation and analysis of drug-resistant kinase alleles has provided insights into the molecular basis of kinase inhibition. Such mutations most commonly alter the so-called 'gatekeeper residue' (a conserved and usually hydrophobic amino acid that limits access to the ATP-binding site). The gatekeeper residue is often found in resistance mutations is remarkably conserved among kinases and drugs. For example, mutations that enlarge this residue provide profound resistance to ATP competitive inhibitors of Abl kinase in CML and EGFR in lung cancer [17,24]. Crystallographic studies reveal that competitive inhibitors of ATP typically occupy a deep pocket conferred by a small gatekeeper residue. When this residue is enlarged, it allows enzymatic activity to be preserved but selectively prevents binding of high-affinity inhibitors.

The identification of a gatekeeper residue empowers rational engineering of drug-resistant kinase alleles, especially since this residue can be readily identified by sequence alignment [25]. Figure 3 illustrates the value of this approach for distinguishing on- and off-target drug effects. In this example, a multitargeted drug can affect 3 different kinases which have different physiologic functions known to be important for cancer. Each of these kinases

mediates different cancer phenotypes and, in sum, inhibition leads to loss of these phenotypes, proliferation arrest, and cell death (Figure 3B). To determine which phenotypes are mediated by the second kinase, the gatekeeper residue can be enlarged, blocking drug binding without altering catalytic activity. If, for example, mitogenic signaling resumes, this would identify kinase 2 as the mediator of this drug effect (this could be measured, for example, by immunoblotting for signaling cascades or cell cycle analysis a short time after drug addition). Individual drug-resistant gatekeeper mutants have been thus exploited to validate on-target effects of individual kinase inhibitors [26].

The same logic can be used to resolve the physiologically relevant targets of other promiscuous kinase inhibitors, by introducing a panel of appropriately modified kinases into cells and selecting for the acquisition of drug resistance (Figure 4). Repeated isolation of clones expressing a particular mutant kinase would strongly suggest that this kinase is either a direct target of the drug or an important mediator of cell survival in the context of drug treatment. Thus, a library of kinases with gatekeeper mutations may be of broad use to identify which are key substrates mediating drug effects.

A recent study exploited a small gatekeeper library to discover key targets of dasatinib, a marketed drug that can affect more than 40 kinases with high affinity. To determine which of these 40 targets mediate the cytotoxic effects of this drug in lung cancer cells, Li *et al.* identified which were most potently inhibited in cells by measuring autophosphorylation. Next, mutations in gatekeeper residues (homologous to Abl T315I) were engineered into each of 13 kinases which most likely mediated the effect of dasatinib on proliferation arrest and apoptosis. This revealed that among the 40 kinases inhibited by dasatinib, 3 members of the Src family (Src, Fyn) mediate drug effects. A similar analysis in glioblastoma cells concordantly revealed the important function of Src and Fyn [27]. We anticipate that a broader panel of gatekeeper mutants may be of general utility for elucidating physiologically significant drug targets (Figure 4).

4. Validating drug targets through engineered analog sensitivity

Analog-sensitive chemical genetics involves directed mutation of a kinase to render it susceptible to inhibition by otherwise biological inert compounds, such as bulky ATP analogs (Fig 1B)[28]. By mutating the gatekeeper residue to a smaller amino acid such as glycine, the binding pocket is enlarged to accommodate a bulky ATP analog as a competitive inhibitor—termed analog sensitive. Since no native protein kinase has a glycine in this position, such inhibitors are too bulky to fit into the ATP binding pockets of any other kinase and thus biologically inactive towards unmodified cells. These bulky ATP analogs do not have any significant effect on proliferation or transcriptional expression profiles in wildtype yeast [28,29]. However, these analogs powerfully block catalytic activity of sensitized kinases. This approach has been widely used to probe kinase function in yeast, and has also proven useful in human cells and transgenic mice. For example, transgenic mice that overexpress an analog sensitive version of CaMKII have memory deficits that are reversed by adding a bulky ATP analog (1-NMPP1) to their drinking water [30].

Analog-sensitive mutations have made important contributions to our understanding of cancer therapies and putative drug targets. For example, as noted above imatinib can block function of KIT and other targets in addition to its primary target, Abl kinase. Which, if any, of these other targets are important for its therapeutic effect? To answer this, Wong and coworkers expressed BCR-ABL wildtype or T315A (analog sensitive) into an IL-3-dependent murine cell line. These BCR-ABL alleles allowed cells to proliferate in the absence of IL-3 signaling unless inhibited with imatinib (for wildtype) or 1-NaPP1 (bulky ATP analog, for T315A). When introduced into mice, these cells replicated

myeloproliferative disease. However simultaneous inhibition of BCR-ABL and KIT with imatinib was more effective than monospecific BCR-ABL inhibition, suggesting that both these targets of imatinib are important for its therapeutic effects.

Other studies have elucidated function of potential cancer drug targets on human cell physiology. This approach is attractive because it potentially allows validation of drug targets in cancer before investing in time and resources in drug discovery, but requires that one replace the endogenous kinase *in vivo* with an analog-sensitive mutant, using either RNAi or gene replacement techniques. For example, Larochelle *et al.* used homologous recombination to mutate both copies of the CDK7 locus (which codes for the sole known Cdk-activating kinase or CAK) in a human colorectal cancer cell line. Acute treatment of the resulting cells with 1-NM-PP1 strongly suppressed Cdk1 and Cdk2 activation and suppressed both the G1/S and G2/M transitions *in vivo* [31]. Such phenotypes were not detected in earlier studies where Cdk7 levels were inadequately reduced by RNAi, and thus reveal novel insights into the control of Cdk/cyclin complexes in higher organisms. In addition, these results suggest that selective inhibitors of Cdk7 will have antiproliferative effects on tumor cells that are similar to broad-spectrum Cdk inhibitors such as flavopiridol. A similar analog-sensitive approach has been used with human Polo-like kinase 1 (Plk1), revealing its crucial roles in both early and late stages of mitosis and cell division [32,33].

In principle, analog sensitivity can be ported to any kinase in the human genome, allowing the construction of a library of tumor cell lines or transgenic mouse models in which all kinases are replaced with an analog-sensitive variant, either through RNAi or gene targeting methods. The resulting cell lines or mice could then be tested for analog-sensitive tumor growth, either in isolation or in combination with other cancer therapies. By focusing on the roughly 500 kinases in the genome, rather than the 10^{60} molecules that populate chemical space (Figure 5), the problem of identifying beneficial drug/target pairings becomes substantially less complex.

5. Outlook

Chemical genetics has the potential to fill in major gaps in our understanding of how current and future anti-cancer therapies work, as well as the reasons they sometimes fail. Ultimately, such information will be crucial in improving these therapies to achieve long-term regression and perhaps even cures in the clinical setting. Lessons learned from the application of chemical genetics to protein kinases may also provide more general insights that can be applied to other classes of oncogenes, including ones currently regarded as undruggable.

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Figure 1. Drug-resistant and analog-sensitive versions of chemical genetics

In chemical genetics, mutation controls a gene product's susceptibility to chemical inhibition. A. Forward genetics. The on- versus off-target effects of a drug are distinguished by constructing (or selecting for) a mutant version of the kinase that is resistant to the drug *in vitro*, and then asking if this mutant renders cells or organisms resistant to the drug *in vivo*. Off-target effects will continue to persist despite reversal of on-target inhibition. B. In analog-sensitive chemical genetics, an individual kinase of interest is deliberately altered (via site-directed mutagenesis) so that its active site can accommodate a normally inactive chemical, such as a bulky ATP analog. This approach is generally applicable to most protein kinases, but requires the availability of methods for replacing the endogenous kinase with the analog-sensitive version[28].

Figure 2. Oncogenic kinases for cancer therapy

Receptor tyrosine kinases (RTKs) are transmembrane proteins that dimerize when bound to ligand. EGFR and ErbB2/Her2 are examples of oncogenic RTKs. Downstream mitogenic signaling cascades include the canonical Ras signaling pathway and the PI3K pathway. These protein kinases typically phosphorylate serine/threonine residues. Here, mitogenic signaling protein kinases are boxed. Oncogenic mutations have been found in AKT and Raf among the protein kinases.

Figure 3. Resistance mutations can be used to identify *in vivo* drug targets

A drug can frequently inhibit multiple protein kinases, making it unclear which mediates important therapeutic effects and which mediate toxicity. This can be evaluated in model systems as shown. A. A system in which 3 protein kinases mediate multiple cancer phenotypes. B. A multitargeted drug can block function of multiple kinases, leading to simultaneous reversal of all phenotypes and cell death. C. Spontaneous or engineered mutations can block drug binding to one kinase, revealing the crucial functions of this individual kinase.

Figure 4. Mapping the physiologically relevant targets of a promiscuous drug

Target kinase inhibitors often display complex spectra of activity when evaluated using in vitro kinase assays. Here, we propose a rescue experiment in which putative target kinases are mutated and introduced into cancer cells of interest. In most cases, kinase mutations will not rescue cells from lethal concentrations of the drug. However, if a drug-resistant version of the target kinase is introduced (here #2), clones will survive the selection procedure.

Using Chemical Genetics in Oncology

Chemicals
N=10⁶⁰

Targets
N=512

Models

Figure 5. Chemical genetics exploits biological context to constrain chemical space
Current drug discovery efforts seek to identify good drugs from chemical space. Chemical genetics allows a focus on the much smaller number of drug targets. Once an important target is validated (shown in green), a drug with sufficient (but not necessarily perfect) selectivity can be developed.

Table 1

Selected oncogenic protein kinases [9]

Kinase	Mechanism of activation	Cancer type	Drugs
ABL	Translocation with BCR	CML, ALL	Imatinib, dasatinib
ALK	Translocation with NPM	Anaplastic Large Cell lymphoma	*
c-KIT	Activating mutation	GI Stromal Tumor	imatinib
AKT1	Activating mutation	Breast, lung, ovarian, colorectal	*
BRAF	Activating mutation and translocation	Melanoma, colorectal, papillary thyroid, lung cancer, cholangiocarcinoma	*
CDK6	Translocation with MLLT10	ALL	*
EGFR	Activating mutation	Lung, glioma	Erlotinib, gefitinib
ERBB2/HER2	amplification	Breast, ovarian, lung, GI	Lapatinib
FGFR2	Mutation	Gastric, lung, endometrial	*
FGFR3	Mutation	Bladder, myeloma, T cell lymphoma	*
FLT3	Mutation	AML, ALL	*
KDR (VEGFR2)	Mutation	Lung cancer, angiosarcoma	Sunitinib, sorafenib
PDGFR α	Mutation, Translocation with FIP1L1	GIST, hypereosinophilic syndrome	imatinib
PDGFR β	Mutation, Translocation with ETV6, others	Myeloproliferative disease, AML, CML	imatinib

* No standard drugs are yet available against this target.

Table 2

Selected cell cycle and mitotic kinases

Kinase	Essential	Functions
Cyclin kinases		
CDK1	Y	M-phase regulation
CDK2	N	S-phase entry
CDK7/CAK	probable	Activate RNA polymerase II, CDK activation
Aurora kinases		
Aurora A	probable	Activation of Plk1, spindle morphology
Aurora B	Y	Destabilize kinetochore-microtubule interactions, Cytokinesis
Polo-like kinases		
Plk1	Y	Mitotic entry, spindle morphology, chromosome arm segregation, cytokinesis
Plk2	N	
Plk3	N	S-phase entry
Plk4/SAK	probable	Centrosome duplication
NIMA kinases		
Nek1		
Nek2	probable	
Nek3-11		
Others		
TTK/Mps1	Y	Mitotic checkpoint
BubR1	Y	Mitotic checkpoint

Table 3

FDA approved inhibitors of protein kinases

Drug	Intended target	Other targets	Suspected target	Kinases inhibited*	Cancer type
Sorafenib	BRAF	PDGFR α	VEGFR1/2	32/11	kidney
Erlotinib	EGFR		EGFR	-/9	Lung
Lapatinib	Her2	EGFR	Her2	3/-	Breast
Sunitinib				109/53	
Imatinib	ABL	c-KIT, PDGFR	ABL, c-KIT	10/19	CML, GIST
Dasatinib	ABL		ABL	61/43	CML
Nilotinib	ABL			-/-	CML
Pazopanib	VEGFR2	c-KIT, PDGFR		23/-	kidney

* number of kinase targets with dissociation constants < 500 nM [16] (number before slash) or inhibited by >80% using in vitro activity assays (Invitrogen SelectScreen; number after slash).