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Kinase Inhibitors — Targeted Personalized Cancer Therapy

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1. Introduction

Cancer arises when a group of cells of a specific tissue type acquires genetic alterations which allow them to grow in an uncontrolled manner eventually invading surrounding tissue and/or traveling to other sites in the body disrupting the normal bodily functions and can result in death. Often the type of acquired genetic alteration in the cancer cell is one which causes the over or under production of a protein important to the regulation of cell growth, or the production of a mutant form of such a protein that is overactive or less active (or not active) or that has a different function than the normal form of the protein. This alteration in expression pattern or altered function contributes to the cancerous state.

The science and art of molecular biology applied to tumor cell lines has provided much information about how mutations contribute to the development of cancers. One group of proteins that are often mutated in cancers are receptor tyrosine kinases. These proteins sit on the cell surface and bind to molecules such as growth factors and result in the growth, maintenance and multiplication of cells. When an activating mutation occurs in a receptor tyrosine kinase the growth factor signal is no longer needed and the cell grows and multiplies uncontrollably, contributing to a cancerous state. While mutations in receptor tyrosine kinases has garnered much attention, and many drugs have been developed targeting them, these kinases are by no means the only relevant kinase family to cancer.

In the past decade there has been rapid growth in the number of FDA approved cancer drugs within the class known as kinase inhibitors. Some kinase inhibitors have become first line targeted therapy for certain tumor types. For example, imatinib (Gleevec) is used effectively to treat Philadelphia chromosome positive (Ph+) chronic myeloid leukemia (CML). The Philadelphia chromosome encodes the mutant BCR-Abl kinase, an oncogenic driver for CML.

Unfortunately, resistance to kinase inhibitors can develop through up-regulation of the target kinase, or mutation of the target kinase resulting in decreased drug binding.

In the most ideal sense of targeted therapy for cancer, kinase inhibitors have the potential to fulfill the goal of personalized cancer therapy. Studies have shown kinase inhibitors targeting specific kinases that drive a proportion of patients' tumors can provide better outcomes for those patients. Combine this outcome with the possibility of mutant selective kinase inhibitors which may leave tissue that expresses wild-type kinase largely unaffected and provide a potentially better safety profile.

2. Kinase structure and function

Protein kinase function was first observed in 1954 by Burnett and Kennedy who discovered an enzyme that phosphorylates casein [1]. Protein kinases are a group of protein enzymes that phosphorylate protein and other targets in the cell in order to modulate these target proteins' function. Protein kinases are often target proteins themselves. Protein kinases catalyze the transfer of a phosphate group from adenosine triphosphate (ATP) to amino acid sidechains that bear a hydroxyl group on target proteins, namely serine, threonine, and tyrosine. The phosphorylation of one of these amino acid sidechains can drastically change the local physicochemical environment as the relatively small polar unionized hydroxyl group is transformed into the relatively large polar ionized phosphate group. This change in the local environment can initiate a conformational change in the newly phosphorylated target protein, in an attempt to find a new low energy conformation, which may modulate the function of the target protein. The phosphorylation event may serve to activate or inhibit target protein enzyme function, or simply modify the target proteins' surface to modulate protein/protein interactions. For example, Src homology 2 (SH2) domain containing proteins recognize certain phospho-tyrosine sequences within protein targets which enable them to perform their adaptor or scaffolding function [2].

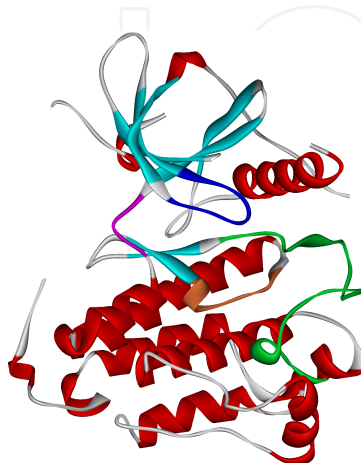


Figure 1. The EGFR kinase domain with color coded structural elements. Cyan; N-terminal lobe. Red; C-terminal lobe. Blue; P-loop. Magenta; hinge. Green; activation loop. Orange; catalytic loop.

Protein kinases are classified into groups based on sequence similarity [3]. The AGC group is named for protein kinases A, G and C. The CaM group is named for calcium/calmodulin-dependent kinases. The CK1 group is named for casein kinase 1. The CMGC group is named for cyclin dependent, mitogen-activated, glycogen synthase, and CDK-like kinases. The STE group is named for sterile phenotype kinase. The TK group is named for tyrosine kinases, and the TKL group is named for tyrosine kinase-like kinases. The groups are further broken down to individual protein kinase families and these families may be further broken down to sub-families. For example, within the TK group is the family called epidermal growth factor receptor (EGFR) tyrosine kinase. The EGFR family includes members EGFR (Her1/ErbB1), Her2/ErbB2, Her3/ErbB3 and Her4/ErbB4. These family members have a high degree of sequence similarity and function.

All protein kinases have a somewhat homologous kinase domain that adopts a common tertiary structure [4]. Figure 1 represents the epidermal growth factor receptor (EGFR) kinase domain and will serve to identify the various secondary structural elements of kinase domain structure and function. Starting from the N-terminus, the basic structural elements of a kinase domain include an N-terminal lobe, composed mostly of beta-sheet structure, and a C-terminal lobe, composed mostly of alpha-helix structure. The two lobes are connected by a hinge region. Of particular interest are the conserved mobile elements of the kinase domain which contribute to kinase function.

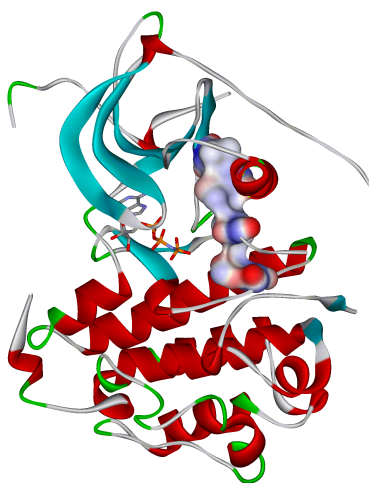


Figure 2. EGFR kinase domain (ribbon) in the active conformation bound to an ATP analog (stick). The active conformation is characterized by alignment of key amino acid sidechains that form a hydrophobic spine (surface representation).

Conformational mobility is essential for kinase function. A number of flexible loop regions are important for the ability to interconvert between active and inactive conformations. These loop regions include the activation loop (A-loop), catalytic loop, nucleotide binding loop (P-loop), and the hinge region. The P-loop is a long loop that joins two anti-parallel beta-strands that are a part of the N-terminal lobe beta-sheet structure of the kinase domain. This loop typically contains a number of glycine residues that are able to access a large range of phi and psi angles

and enable considerable conformational flexibility. In the inactive conformation the P-loop may be largely disordered. In the active kinase conformation the P-loop may resemble more of a classic beta-turn and is able to interact with the triphosphate group of bound ATP. The A-loop includes one or more tyrosine residues that when phosphorylated induce the conversion from the inactive to the active kinase conformation. The A-loop is long, containing around 27 amino acid residues that may fold into a number of isolated single turn helices. The A-loop has a conserved DFG sequence, where in the non-phosphorylated inactive kinase conformation the phenylalanine sidechain is oriented toward the triphosphate binding area of the ATP binding site, the so called DFG-out conformation. In the phosphorylated active kinase conformation, the DFG phenylalanine sidechain is oriented toward the interior of the protein, the so-called DFG-in conformation [5-7]. While the DFG orientation related to active and inactive kinase conformations is clear in some cases it is not clear in others, and is not as general in differentiating active and inactive conformations as first thought. It has subsequently been found through surface comparison of x-ray derived crystal structures of both active and inactive forms of specific kinases, that the assembly of a hydrophobic spine through sidechain interaction of discontinuous amino acids within the N-lobe, C-lobe and activation loop regions offers a better structural definition of kinase activation for all kinase families [8]. Figure 2 demonstrates the hydrophobic spine assembly of a representative kinase. Both active and inactive kinase conformations are able to bind ATP. However, only the active kinase conformation can perform the kinase function on substrates.

Protein kinases are important to many biological processes, as is evidenced by the more than 500 kinases encoded in the human genome. Kinases are involved in processes ranging from modulation of intracellular trafficking of proteins, to assembly of complicated multiprotein complexes, to gene expression. Kinase modulation of gene expression through signal transduction is of particular interest for cancer therapy.

The underlying cause of cancer is genomic instability. The emergence of a tumor cell typically requires multiple mutations in the genome that both activate oncogenes and inactivate tumor suppressor genes. These mutations synergize to transform a cell to a state of uncontrolled growth, proliferation, and enhanced survival. Mutations and/or epigenetic alterations that increase expression of, or produce constitutive active forms of some kinase proteins are known to drive the proliferation and/or survival of some tumors [9]. The types of kinases that are often mutated in tumor cells, although not exclusively, are found in signal transduction pathways. These pathways are kinase cascades that transmit extracellular signals to the nucleus and ultimately affect gene expression patterns. When a kinase in such a pathway is mutated where it is constitutively active, the extracellular signal is no longer needed and the pathway is always active and not regulated. Alternatively, epigenetic alterations or an increase in copy number of a protein kinase gene may cause overexpression of the kinase to increase activation of a signaling pathway. For example, a significant portion of non-small cell lung cancers (NSCLC) over express or have EGFR mutations. This causes over active signaling through one or more of the Ras/Raf/ERK, PI3K/AKT/mTOR, and STAT pathways that lead to increased growth, proliferation, motility and survival of the tumor cells.

The KIT receptor tyrosine kinase, or stem cell factor receptor, is a good example for representing the different types of mutations that occur in kinases that contribute to the development of cancer. KIT possesses an extracellular domain, a juxtamembrane domain, and a kinase domain. Point mutations have been observed to occur in all domains of KIT [10]. The most common mutations occur in the juxtamembrane domain followed by the extracellular domain, and finally the kinase domain. Within the kinase domain mutations occur within the ATP binding site and the activation loop. Multiple mutations may occur within a tumor cell. All of these mutations produce constitutive active KIT. For example, juxtamembrane mutations result in the disruption of the autoinhibited form of the kinase, which results in a similar conformation to the phosphorylated activated kinase [11]. Additionally, a KIT isoform with a GNNK insert in the extracellular domain has been identified that, once phosphorylated remains so for longer than the isoform that does not contain this insert, increases survival of myeloma cells [12, 13]. Overexpression of wildtype KIT may also drive tumor development. Overexpression may be due to an increase in gene copy number and/or hypomethylation of the gene promoter region.

The link between the development of some tumors, and aberrant modulation of certain protein kinases-either wildtype or mutant- has been established. Some of these aberrations, such as the BCR-Abl protein, represent driver mutations that have led to the successful translation of experimental protein kinase inhibitors into the clinic for therapeutic treatment of cancer patients.

3. Kinase inhibitors

The natural product staurosporin was one of the first small molecules discovered that inhibits the function of protein kinases [14]. Staurosporin is a non-selective inhibitor and is not amenable to development as a drug. However, one staurosporin-like molecule, midostaurin, is in clinical development for oncology indications such as indolent systemic mastocytosis [15].

Imatinib, the first marketed kinase inhibitor for treatment of cancer, was discovered by scientists at Ciba-Geigy in the 1990s. It was determined that imatinib inhibited the function of the BCR-Abl fusion kinase that drives chronic myeloid leukemia [16]. Since this time (and perhaps before) the primary rational approach to design of kinase inhibitors has been to target the ATP binding site. The first experimentally determined structure of a small molecule bound to the ATP binding site of a kinase appeared in the year 2000 [17]. This structure was groundbreaking, in that it showed imatinib to be buried deeply in the ATP binding site of the kinase thus locking it in an inactive conformation. This structure provided data that could be used for a structure based design approach of future kinase inhibitors. The structure revealed a pose of imatinib in the ATP binding site that is today designated the type II binding mode.

Clinically used ATP site binding direct kinase inhibitors fall within two categories that describe their binding modes. Type I inhibitors may bind both active and inactive conformations of a particular kinase target. Type II kinase inhibitors bind only inactive conformations of a kinase. Both type I and II inhibitors bind in the ATP binding site and make use of hydrogen bonds to

the amide backbone of hinge region amino acid residues. The difference between type I and II inhibitors is that type II inhibitors typically penetrate deeper into the pocket accessing what is termed an allosteric binding site, the effect of which is to displace the C-helix and locking the kinase in an inactive conformation. Knowledge of binding geometries has been applied to kinase inhibitor design projects.

The majority of kinase inhibitor design projects have focused on ATP competitive inhibitors targeting the ATP binding site. The popularity of such programs have spurred the growth of contract research screening services that screen potential inhibitors in a high throughput format for binding to the ATP binding site and/or inhibition of target kinase function [18]. Design programs may be conducted in a variety of manners from high throughput screening of compound libraries to fragment based and/or structure guided design. One approach is to start from known privileged fragments or templates - chemical moieties that appear in numerous kinase inhibitors - such as the quinazoline ring system [19]. The quinazoline ring system appears in many experimental kinase inhibitors, clinical candidates (tandutinib), and kinase inhibitor drugs such as afatinib, vandetinib, lapatinib, erlotinib and gefitinib (see Table 1).

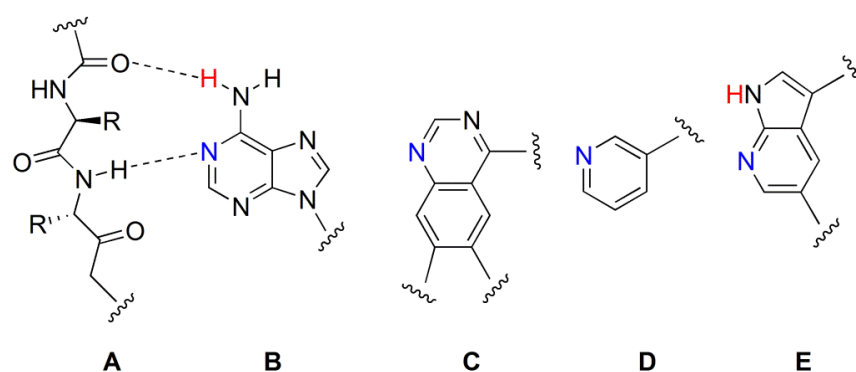


Figure 3. Kinase hinge peptide backbone (A) hydrogen bonding pattern to ATP heterocycle (B) and other heterocycles found in drug molecules. (C) quinazoline, e.g. erlotinib. (D) pyridine, e.g. imatinib. (E) pyrrolopyridine, e.g. vemurafenib. Dashed lines represent hydrogen bonds.

It appears that a unifying characteristic of kinase inhibitors that bind to the ATP binding site is a heterocyclic moiety that serves as a mimic of the adenine ring system and forms Van der Waals contacts with hydrophobic groups of the floor and ceiling of the ATP binding site as well as one or more hydrogen bonds with the amide backbone of hinge region amino acid residues. Figure 3 describes how ATP and some heterocycles found in kinase inhibitors form their hydrogen bonding patterns to the hinge region. Because the ATP binding site is conserved among kinases, kinase inhibitors tend to be promiscuous and inhibit numerous kinases other than their primary target. How the final kinase inhibitor is adorned about its hinge binding moiety contributes to its overall shape and possible intermolecular interactions, ultimately determines its degree of selectivity for its intended target.

Another unifying theme of kinase inhibitors is that their binding to their kinase targets displaces the alignment of the hydrophobic spine that is formed by kinase activation [8].

Besides the occupation of the ATP binding site by the kinase inhibitor, disallowing binding and use of ATP, the kinase inhibitor disrupts the formation of the hydrophobic spine necessary for attainment of the active conformation (Figure 4). This observation leads to the inevitable question of whether inhibitors, that disrupt the hydrophobic spine, but do not bind to the ATP binding site, may be developed.

Considering the mountain of published research and plethora of X-ray diffraction and NMR derived structures of kinase inhibitors bound to their target kinases, the design of kinase inhibitors is fairly well understood. The remaining challenges for drug designers, in the area of kinase inhibitors, are concerned with selectivity. Selectivity is a potential problem for kinase inhibitors, thus far, because most target the highly conserved ATP binding site. Targeting a single family of kinases, let alone a single member of a family, is difficult because of the inherent promiscuity of this drug class. An even more difficult challenge is targeting a cancer relevant mutant kinase selectively with respect to the wild type kinase. The selective targeting of a mutant kinase that drives the proliferation and survival of a patients' tumor has great promise not only in potential survival benefit but also reduction in drug side effects.

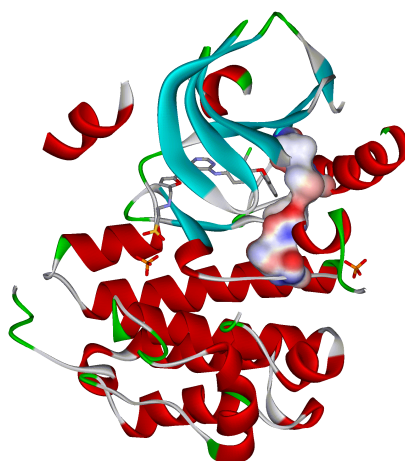


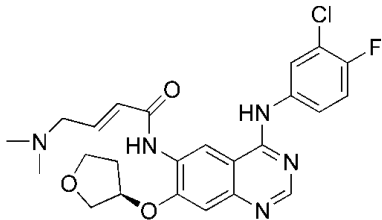
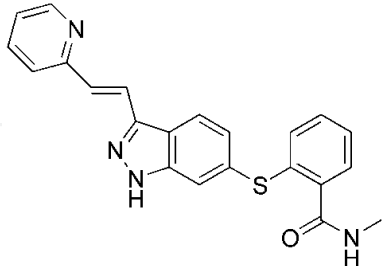
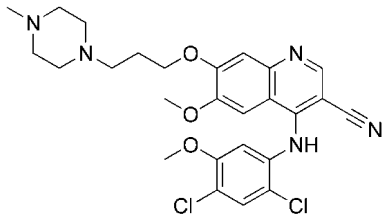
Figure 4. EGFR kinase domain (ribbon) bound to kinase inhibitor lapatinib (stick). Note that the amino acid sidechains that form the hydrophobic spine (surface) are bowed out of alignment.

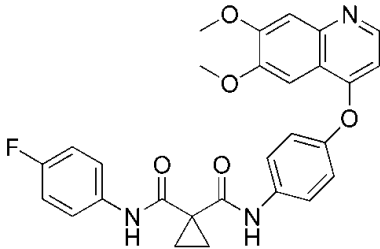
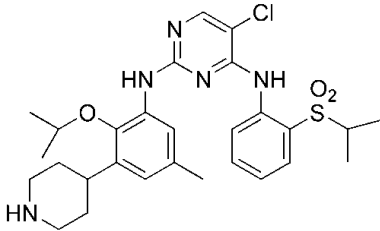
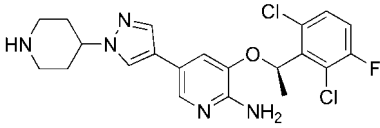
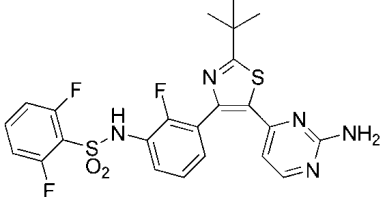
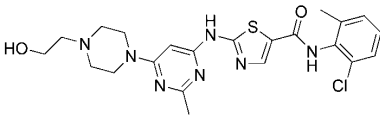
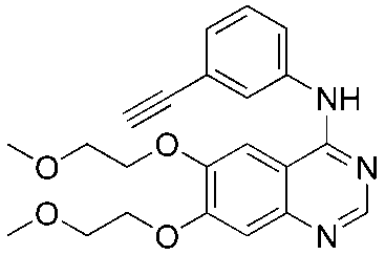
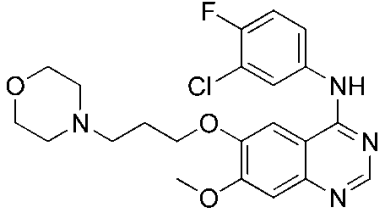
Common side effects caused by kinase inhibitors include rash, fatigue, and gastrointestinal disturbances such as nausea, vomiting, diarrhea, or constipation. For example, patients receiving therapy with EGFR inhibitors may experience severe acneiform eruption and diarrhea which may be dose limiting [20]. Additionally, the EGFR inhibitor gefitinib has been associated with interstitial lung disease, especially in patients with underlying pulmonary diseases [21]. The more promiscuous kinase inhibitors such as sunitinib and sorafenib exhibit some cardiotoxicities such as hypertension, LVEF and QT prolongation [22]. Additional less severe side-effects may be experienced by patients receiving kinase inhibitors such as hair depigmentation which accompanies KIT inhibitors [23]. Even though kinase inhibitors are considered targeted therapy the patient will experience side effects that can be associated with the mechanism of action, off target effects and intrinsic chemical effects. Almost as common as side effects are associated with anticancer drugs so is tumor resistance. And just as other

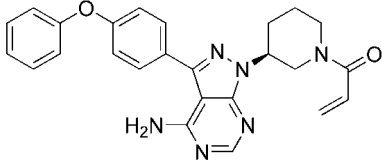
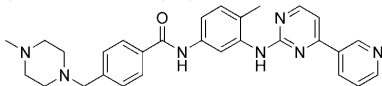
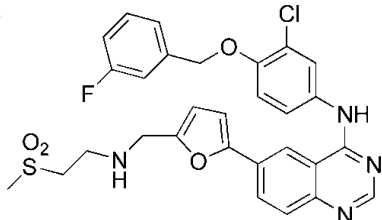
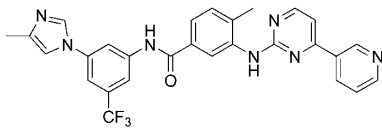
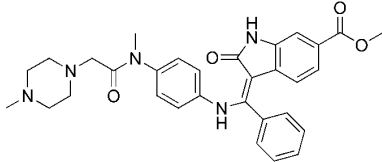
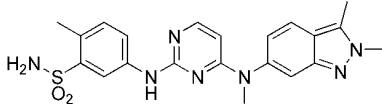
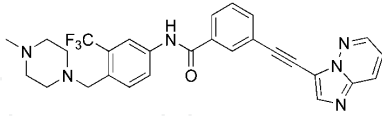
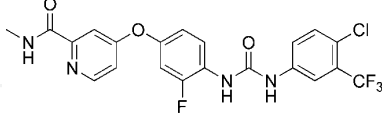
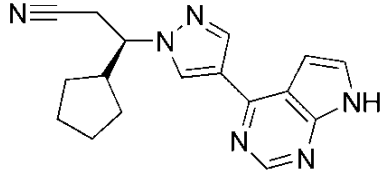
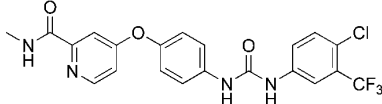
anticancer drugs may encounter tumor resistance, a kinase inhibitor may also encounter resistance either acquired or intrinsic.

Tumor resistance to anticancer drugs can be a challenging battle even though a lot has been learned about the mechanisms that allow tumor cells to escape the chemical assault. One major mechanism of tumor resistance to kinase inhibitors is mutant forms of the target kinase, either preexisting or acquired during or after treatment, that reduce the kinase inhibitors binding affinity. Early clinical experience with imatinib identified patients that developed resistance after an initial response to the drug due to mutation of the ACR-Abl kinase that prevented imatinib binding [24, 25]. Experience with KIT inhibitors has revealed drug resistant mutations in this kinase [10]. Pharmacokinetic mediated tumor resistance to kinase inhibitors include, polymorphisms in drug metabolizing enzymes and transporters. For example, CML patients with low human organic cation transporter-1 (hOCT1) activity have suboptimal response to imatinib but not to nilotinib [26, 27]. Other mechanisms of tumor resistance to kinase inhibitors may include unique features of the tumors microenvironment and activation of alternative signaling pathways. For a more comprehensive discussion of tumor resistance to kinase inhibitors the reader is directed to the references [28, 29].

Since the year 2001 there have been no less than 24 small molecule, ATP site binding kinase inhibitors approved by the USFDA for oncology indications (Table 1). As the concept of oncogene addiction is explored more and a better understanding of the role of kinases in oncology is realized, the class of kinase drugs may be ideally situated to have a profound effect on personalized cancer medicine.

Structure	Generic Name	Target(s)	Indication(s)
	afatinib	EGFR	NSCLC
	axitinib	VEGFR 1/2/3	RCC
	bosutinib	BCR-Abl, Hck, Lyn, Src	CML

Structure	Generic Name	Target(s)	Indication(s)
	cabozantinib	RET, MET, VEGFR 1/2/3, KIT, TRKB, Flt-3, AXL, TIE-2	MTC
	ceritinib	ALK, IGF-1R, InsR, ROS1	ALK-positive NSCLC
	crizotinib	ALK, c-Met, ROS	ALK-positive NSCLC
	dabrafenib	B-RAF	melanoma
	dasitinib	BCR-Abl, SRC, LYN, YES, FYN, KIT, EPHA2, PDGFRb	CML
	erlotinib	EGFR	NSCLC, pancreatic cancer
	gefitinib	EGFR	NSCLC

Structure	Generic Name	Target(s)	Indication(s)
	ibrutinib	BTK	CLL, mantle cell lymphoma
	imatinib	BCR-Abl, PDGFR, KIT	CML, ALL, GIST, ASM
	lapatinib	EGFR, ERBB2	Breast cancer
	nilotinib	BCR-Abl, PDGFR	CML
	nintedanib	VEGFR, FGFR, PDGFR	IPF
	pazopanib	VEGFR, PDGFR, FGFR, KIT, Lck, FMS, ITK	RCC, soft tissue sarcomas
	ponatinib	BCR-Abl, VEGFR, PDGFR, Src, KIT, Flt3, RET, Tie2	CML, Ph+ All
	regorafenib	VEGFR, BCR-Abl, B-Raf, PDGFR, FGFR, Tie2	CRC
	ruxolitinib	JAK	myelofibrosis
	sorafenib	Multi-kinase inhibitor	RCC, DTC

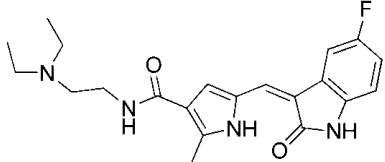
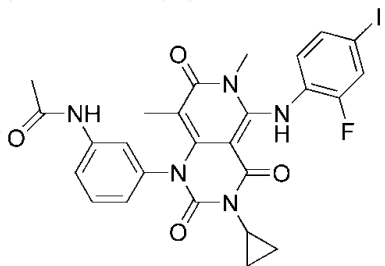
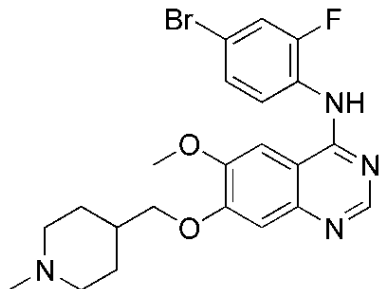
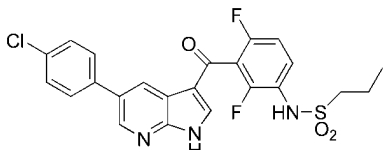
Structure	Generic Name	Target(s)	Indication(s)
	sunitinib	Multi-kinase inhibitor	RCC, GIST, pancreas cancer, neuro-endocrine tumors
	trametinib	MEK	melanoma
	vandetanib	EGFR, VEGFR, RET, Brk, Src, Tie2	MTC
	vemurafenib	Raf kinases	melanoma

Table 1. USDA approved kinase inhibitors for oncology indication.

4. Personalized medicine and kinase inhibitors

The basic concept of personalized cancer medicine is to match the patient with an appropriate therapeutic regimen that will provide the best outcome [30]. This can be accomplished on a variety of different levels. With regard to a pharmacological approach, the traditional way patients are assigned to therapy is based on tumor type, that is, what anatomical site at which the tumor resides and/or what type of histopathology the tumor cells derive from. Once these pieces of information were obtained the patient would be assigned a pharmacotherapy regimen that provided the best statistical outcome based on collective experience. This approach has worked as well as can be expected, and exceedingly well in some tumor types considering the diseases' high mortality. However with modern scientific advances and strive toward better outcomes, an even more personalized approach has emerged.

A modern approach to personalized cancer pharmacotherapy can be considered on three basic levels which can be applied individually or combined. These three levels are the patient's

pharmacogenetics, the tumor's genomics and/or proteomics, and the tumor's response to drug exposure. Patient pharmacogenetics refers to the patient's genetics that are relevant to effects on pharmacokinetics of drugs, for example, polymorphisms in drug metabolizing enzymes. Tumor genomics and/or proteomics refers to specific genetic abnormalities that effect specific gene expression and is thought to contribute to tumor development or maintenance. The application of any or all of these pieces of information may improve the outcome of pharmacotherapy for the patient.

The application of pharmacogenomics to personalized therapy can be exemplified by application to tamoxifen therapy for the breast cancer patient. Tamoxifen acts as an anti-estrogen on breast tissue and is used for treatment of estrogen receptor positive (ER+) breast cancer. It has been determined that tamoxifen is converted in vivo to 4-hydroxytamoxifen, which is a much more active anti-estrogen agent. It was subsequently found that this transformation is accomplished by the drug metabolizing enzymes cytochrome P-450 (CYP) isoforms 2D6 and 2C19 [31]. Patients that were receiving tamoxifen therapy and concurrently receiving selective serotonin reuptake inhibitor (SSRI) drugs, known to inhibit CYP 2D6, experienced poor outcomes because of the failure to convert tamoxifen to 4-hydroxytamoxifen. A significant percentage of the population carry a genetic polymorphism in the CYP 2D6 gene which results in poor metabolism with respect for the CYP 2D6 isoform and will not receive the full benefit of tamoxifen therapy [32]. Consequently a readily available genomic test is available for patients that can identify those who are not good candidates for tamoxifen therapy, because they have a specific 2D6 polymorphism, so that they can be directed to alternatives.

Advances in genomics and proteomics have enabled selection of patients that may benefit from targeted therapies for certain tumor types. Analysis of tumor cells on the protein level using immunohistochemistry (IHC) can identify cells that express relevant protein targets. An example of the application of this approach is the detection of c-erbB2 (Her2/Neu) receptor in breast cancer patients which can direct them toward trastuzumab therapy. Specific gene mutations in tumor cells may be detected using techniques of RT-PCR, DNA microarray, and fluorescence in situ hybridization (FISH) that can be used to direct patients to therapy that may be beneficial. For example, a study demonstrated the utility of screening non-small cell lung cancer patients for amplification of the epidermal growth factor receptor (EGFR). Patients with amplified EGFR receiving an EGFR kinase inhibitor (gefitinib) had longer median progression free survival (PFS) and overall survival (OS), compared to those who did not have amplified EGFR [33, 34]. Furthermore, of those patients who responded to gefitinib therapy 77% had EGFR gene amplification, whereas only 33% of non-responders had EGFR gene amplification. This study demonstrates the utility of identifying patients with mutations that drive tumor growth and survival and matching them with appropriate targeted therapy. The study also highlights the shortcoming of single gene determination directed therapy in that 33% of non-responders also had EGFR amplification.

A potentially more invasive and perhaps technically challenging approach is to directly test samples of the patient's tumor against available drugs. This approach of using personalized xenografts to direct patient therapy was demonstrated in a patient with advanced pancreatic patient [35]. After initial surgery metastases were discovered and adjuvant gemcitabine

therapy failed to halt progression. A personalized mouse xenograft model was developed from the patient's tumor tissue and was found to respond to the DNA alkylating agents, mitomycin C and cisplatin. The patient was assigned to mitomycin C treatment and subsequently cisplatin and achieved a partial response with duration of 50+ months. Genomic analysis of the patient's tumor tissue revealed inactivation of the PALB2 gene, which is involved with repair of double strand DNA breaks. It seems logical that the patient's tumor responded to the DNA alkylating agents that would cause double stranded DNA breaks.

The genomic approach has been applied to kinase inhibitors from the beginning of their introduction to the arsenal of pharmacotherapy options. Imatinib, the first kinase inhibitor to be marketed is targeted at a specific genomic alteration, a chromosomal translocation producing the Philadelphia chromosome (Ph) that expresses a mutant gene product, the BCR-Abl kinase. This kinase is constitutively active, and is the driver of nearly all chronic myeloid leukemias (CML). Therefore, patients with CML and are Ph+ can be matched to imatinib therapy. Imatinib therapy has been fairly successful for CML patients. It has been shown that patients who achieve complete cytogenic response at 2 years on imatinib therapy tend to maintain the durable response and do not have mortality significantly different than the general population [36].

Because the effectiveness of kinase inhibitors require that the target kinase to be a driver of, expressed in, or aberrantly expressed in, or be mutated in the tumor cells, it is important to know the status of the target within the specific patient's tumor in order to best assign the patient to kinase inhibitor therapy. To this end specific diagnostic tests have been developed to help guide selection of kinase inhibitor therapy. Indeed, twelve of the approved kinase inhibitors' prescribing information assume diagnostic testing be performed, and of those, four require a diagnostic test for prescription. Table 2 lists six such diagnostics recognized by the USFDA [37].

5. Emerging kinase targets

Because kinase enzymes are involved in numerous biological processes it is not surprising that many of them have become therapeutic targets for various disease states including cancers. As noted above, drug development of kinase inhibitors for oncology indications has accelerated in the last decade with no sign of slowing. This acceleration has been spurred by emergent technologies and advances in molecular and systems biology, proteomics, and genomics. The continued advancement of understanding of the molecular changes that occur in the development of a cancer has helped identify likely therapeutic targets. Some of the more recently identified targets include the BTK, CDK8, and DNA-PK.

Although a Bruton's tyrosine kinase (BTK) inhibitor (ibrutinib) was recently approved for clinical use, BTK is a relatively new kinase target. BTK plays a role in B-cell receptor signaling, proliferation, differentiation, and survival. Ibrutinib works by irreversible inhibition of BTK through covalent modification of the enzyme. In a clinical trial in mantle cell lymphoma (MCL) patients, who had three prior therapies, an overall response rate of 69% and a progression free

survival of 13.9 months were observed. Ibrutinib is approved for second line treatment of MCL and chronic lymphocytic leukemia (CLL) [38].

Drug (generic name)	Diagnostic Product	Diagnostic Product Use
afatinib	Therascreen EGFR RGQ PCR Kit	Detects EGFR exon 19 deletions and exon 21 substitution mutations. Intended for selection of NSCLC patients who may benefit from afatinib therapy.
crizotinib	VTSIS ALK Break Apart FISH Probe Kit	Detection of ALK gene rearrangements. Prescription use only, intended for selection of NSCLC patients eligible for crizotinib treatment.
dabrafenib and tramatenib	THxID BRAF Kit	Detection of BRAF(V600E) and (V600K) mutations in melanoma tissue. Intended to aid selection of melanoma patients who may benefit from treatment with dabrafenib and/or trametinib.
erlotinib	Cobas EGFR Mutation Test	Detects EGFR exon 19 deletions and exon 21 substitution mutations. Intended for selection of patients with metastatic NSCLC who may benefit from afatinib therapy.
imatinib mesylate	DAKO C-KIT PharmDx	Quantitative c-Kit detection in GIST and normal tissues. Intended to aid selection of GIST patients who may be eligible for imatinib mesylate treatment.
vemurafenib	COBAS 4800 BRAF V600 Mutation Test	Detection of BRAF (V600E) mutation in melanoma. Intended to aid selection of melanoma patients who may benefit from vemurafenib therapy.

Table 2. US FDA Cleared or Approved In Vitro Companion Diagnostic Tools for Kinase Inhibitors.

Cyclin dependent kinase 8 (CDK8) was recently identified as a colorectal cancer oncogene [39]. It was discovered that 47% of colorectal cancer samples demonstrated copy number gain of the chromosomal region carrying the CDK8 gene. It has also been shown that 76% of colorectal cancers showed positive expression of nuclear CDK8, and that increased positive expression

rate correlated with increased clinical stage [40]. Additionally, expression of CDK8 transformed NIH 3T3 cells, however a kinase inactive version did not. Experimental knockdown of CDK8, in colorectal cancer cell lines with high CDK8 expression levels, induced a decrease in cell proliferation. While most CDKs regulate cell cycle progression CDK8 and some others play a role in transcription regulation. Assembly of CDK8 with other key protein partners forms a mediator complex which can activate β -catenin dependent transcription. Furthermore it was shown that CDK8 function represses E2F1 activity, a known negative regulator of β -catenin [41]. Of note is the fact that knockdown of CDK8 in some colorectal cancer cell lines does not completely diminish β -catenin levels, which suggests that other genetic determinants of resistance must be identified to exclude patients that would not benefit from a future CDK8 targeted therapeutic.

DNA-dependent protein kinase (DNA-PK) is another emerging oncology kinase target. Being that the development of cancer is due to genomic instability that results in the acquiring of genetic mutations that drive tumor formation, it should be no surprise that tumor cells may have defective DNA repair pathways that result in a mutator phenotype. It is no wonder that the first class of anti-cancer drugs were DNA alkylating agents that cause irreparable damage to DNA of tumor cells, although with severe side effects and risk of secondary tumors arising from DNA damaged normal cells. A less devastating targeted approach to induce irreparable DNA damage in tumor cells, while sparing normal cells, is to target still operating DNA repair pathways that are critical to the tumor cells survival. In other words disable a DNA repair pathway that will synergize with an already disabled pathway. For example, DNA-PK is critical for repairing double strand DNA breaks through the non-homologous end joining (NHEJ) pathway. A second pathway for repairing double strand breaks is the homologous recombination pathway, which is impaired in tumor cells with mutations in genes such as BRCA1 and BRCA2 as well as others. Targeting tumors with impaired homologous recombination by inhibiting the NHEJ pathway with DNA-PK inhibitors should, in theory, cause activation of cell death pathways as double strand DNA-breaks build-up in the cells [42, 43]. Normal cells should be able to withstand the assault due to both pathways being operable.

6. Summary

Kinase inhibitors have enormous potential to facilitate improved outcomes for some cancer patients especially in the context of personalized medicine. The personalized approach that implements genomic analysis to identify potential driver kinases in patient tumor samples will enable matching the patient with the best kinase inhibitor for the best outcome. The identification of drug resistant mutations in the targeted kinase will be critical in order to avoid treatment that is not likely to be beneficial to the patient. Also critical, is the identification of relevant polymorphisms in drug metabolizing enzymes and transporters that may affect pharmacokinetics of the kinase inhibitor, in order to assure adjustments are made to achieve optimum drug exposure. These considerations along with proper management of side effects can maximize patient benefit from targeted personalized cancer therapy with kinase inhibitors.

Nomenclature

ALL-acute lymphoblastic leukemia; ASM-aggressive systemic mastocytosis; CLL-chronic lymphocytic leukemia; CML-chronic myeloid leukemia; CRC-colorectal cancer; DTC-differentiated thyroid carcinoma; GIST-gastrointestinal stromal tumor; IPF-idiopathic pulmonary fibrosis; MTC-medullary carcinoma of the thyroid; NSCLC-non-small-cell lung cancer; RCC-renal cell carcinoma.

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