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Advances of small molecule targeting of kinases

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Abstract

Reversible protein phosphorylation regulates virtually all aspects of life in the cell. As a result, dysregulation of protein kinases, the enzymes responsible for transferring phosphate groups from ATP to proteins, are often the cause or consequence of many human diseases including cancer. Almost three dozen protein kinase inhibitors (PKIs) have been approved for clinical applications since 1995, the vast majority of them for the treatment of cancer. According to the NCI, there are more than 100 types of cancer. However, FDA-approved PKIs only target 14 of them. Importantly, of the more than 500 protein kinases encoded by the human genome, only 22 are targets for currently approved PKIs, suggesting that the reservoir of PKIs still has room to grow significantly. In this short review we will discuss the most recent advances, challenges, and alternatives to currently adopted strategies in this burgeoning field.

The human genome, which may contain as few as 19,000 genes [1], encodes 538 protein kinases [2], representing almost 3 % of the total. Protein kinases are responsible for phosphorylating approximately one third of all proteins [3]. Unlike other post-translational modifications, phosphorylation is reversible and, in most cases transient, because phosphate groups can readily be removed by protein phosphatases. This mechanism greatly enhances the genome's plasticity, and it can regulate protein function in virtually every imaginable way: To name a few examples, it can stimulate or inhibit enzymatic activity, protein degradation, or relocation within the cell. In many human diseases protein kinases are dysregulated, particularly in cancer [4]. Since most protein kinases stimulate cell growth and proliferation, cell survival and migration, they can, when overexpressed, amplified or constitutively active, assume oncogenic properties. It is thus not surprising that in the last few decades enormous efforts have been devoted to developing small molecules that specifically or selectively inhibit protein kinases. Initially, these efforts were hampered by two perceived difficulties: First, given that protein kinases share similar ATP-binding sites, it was considered impossible to develop compounds that inhibit just one kinase. Second, it was thought that these compounds would have to compete with millimolar ATP concentrations inside cells [5]. Eventually potent and specific kinase inhibitors could be developed, and the fact that many PKIs inhibit more than one kinase may actually be advantageous in cancer,

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since this would possibly allow the drug to be used in several types of cancer and prevent acquired drug resistance [5].

Kinase inhibitors are broadly classified as exclusively occupying the ATP-site (Type I), concurrently occupying the ATP site and an adjacent allosteric site (Type II) or solely occupying an allosteric site (Type III). The first kinase inhibitor that was approved for clinical use (1995 in Japan) is the ROCK inhibitor fasudil for treating cerebral vasospasms [2]. As of April 2017, 35 small molecule PKIs have been approved for clinical use, 31 of which are used in cancer therapy (Fig. 1). Sixteen of the originally intended 22 targets are tyrosine kinases. The subject of PKIs has been discussed, in great detail, in excellent recent reviews [5–11]. Here, we will briefly review the most recent advances in this field and its remaining challenges.

Reversible ATP site directed inhibitors

Among receptor tyrosine kinase inhibitors the pan-VEGFR inhibitor lenvatinib [12] was approved in 2015 for thyroid cancer and in 2016 for renal cell carcinoma in combination with the mTOR inhibitor everolimus (Fig. 2). Significant success has been reported in the development of serine-threonine specific kinase (STK) inhibitors, particularly of cyclin-dependent kinases (CDKs). Numerous studies during the early to mid-1990s have suggested a crucial role for CDK4 in cell cycle progression, particularly from G1 to S-phase. Key components of this event are: CDK4 with its partner Cyclin D and the tumor suppressors RB and p16Ink4 (for a review see [7]). The most important function of CDK4/cyclin D appears to be the initial step in sequential inactivating phosphorylations of the RB protein, which in turn leads to the expression of proteins necessary for DNA replication. At the same time, it was already realized that many cancers overexpress either D-type cyclins or CDK4, or they lack functional p16Ink4 or RB tumor suppressors. This and other observations led to the suggestion that ultimately cancer is a disease of the cell cycle. Using mouse models of mammary tumorigenesis, a crucial role of CDK4/cyclin D1 was demonstrated in a series of elegant studies: Thus, knock-in mice expressing a mutant cyclin D1 unable to activate CDK4/6 and CDK4-null mice are resistant to ErbB2-induced breast cancer [13,14]. More recently, it was shown that ablation of cyclin D3 or pharmacologic inhibition of CDK4 results in the selective killing of cancer cells without affecting normal tissues in mice carrying acute lymphoblastic leukemia [15]. It is nonetheless striking that it took until 2015 to gain approval for the first CDK inhibitor (palbociclib, targeting CDK4/6) in the treatment of metastatic breast cancer [10]. The latest addition to the portfolio of approved PKIs, ribociclib, is also a CDK4/6 inhibitor [7]. Like palbociclib, ribociclib inhibits RB phosphorylation and causes G1 arrest in neuroblastoma cells [16]. Palbociclib and ribociclib are the only STK inhibitors approved since 2015. Another potent CDK4/6 inhibitor is abemaciclib (phase III) which has recently been shown to cause tumor regression in mouse models of several cancers including melanoma [17]. The success of CDK4/6 inhibitors also highlights the importance of outcome predictions to select patient populations that are likely to respond to the given drug. The inhibition of CDK4 is only efficacious in cells and patients that express functional RB [18]. Several inhibitors of other kinases involved in cell cycle progression are being evaluated in clinical trials, e.g. AZD1775 targeting Wee1 [19], alisertib targeting Aurora A [20] and LY2603618 targeting CHK1 [21]. Recent inhibitors of

signaling kinases in clinical trials include the JAK1/JAK2 inhibitor baricitinib, an analogue of the FDA approved JAK2 inhibitor ruxolitinib, for refractory rheumatoid arthritis [22,23] and the pan-PI3K inhibitor buparlisib for advanced HR+/HER2 endocrine-resistant breast cancer [24,25] and other cancers [26–28]. In April 2017, the FDA approved the pan-kinase inhibitor midostaurin, a staurosporine analogue, for the treatment of newly diagnosed acute myeloid leukemia (AML) with FLT3 mutation [29].

Reversible allosteric inhibitors

For the longest time, PKIs have exclusively been functioning as ATP-competitive inhibitors. More recently, the field has also seen the advent of inhibitors targeting hydrophobic pockets outside the ATP binding site.[30] To date, there are just three FDA-approved allosteric inhibitors, the MEK1/2 inhibitor trametinib and the macrocyclic rapamycin analogs everolimus and temsirolimus targeting mTOR. While first generation catalytic-site ABL1 kinase inhibitors such as imatinib have led to a shift in the treatment of chronic myeloid leukemia (CML), second-generation inhibitors such as nilotinib were significantly active against imatinib-resistant mutants and showed superior outcomes [31]. Recently a research group from Novartis reported on the development of a novel potent and selective allosteric inhibitor of BCR–ABL1 that binds to the myristoyl pocket [32]. ABL001 (asciminib) is a functional antagonist of BCR-ABL1 by inducing structural changes to the N terminus of ABL1 resulting in an enzymatically inactive kinase conformation. Acquired resistance was observed with single-agent therapy in mice, whereas the combination of asciminib and nilotinib led to complete disease control and eradicated CML xenograft tumors without recurrence after the cessation of treatment. These findings suggest that a combination treatment of catalytic and allosteric site inhibitors of BCR-ABL1 might lead to disease eradication and treatment-free remissions.

Covalent ATP site directed inhibitors

Many kinases have an exposed cysteine side chain in the ATP site that could be targeted for covalent reaction with compounds harboring an electrophilic Michael Acceptor in the right position [33–35]. Such covalent inhibitors are designed as affinity labels that interact with the target kinase in a two-step reaction, in which the formation of an irreversible enzyme-inhibitor complex is preceded by a rapidly reversible collision complex. In 2013, afatinib (targeting EGFR) and ibrutinib (targeting BTK) became the first FDA approved covalent kinase inhibitors. Another covalent EGFR inhibitor, osimertinib, was approved in 2015 for the treatment of non-small-cell lung cancer (NSCLC) characterized by the EGFR T790M mutation [36,37]. Novel covalent inhibitors of EGFR-T790M based on pyrazolopyrimidines[38] and indazoles[39] have been reported recently, and other inhibitors such as rociletinib are in late phase clinical trials for non-small cell lung cancer (NSCLC) [40]. Gray and colleagues developed novel covalent inhibitors to target specific kinase isoforms including JAK3 of the JAK family [41], as well as CDK7 [42,43] and CDK12 [44] which are critical for the transcriptional machinery to sustain the oncogenic state. Compounds THZ1 (targeting CDK7) and THZ531 (targeting CDK12) are unique in that they have been designed to target a Cys residue outside the ATP site, which provides new means of achieving covalent selectivity. To avoid potential off-target effects of irreversible

acrylamide-based kinase inhibitors by forming permanent covalent adducts with cysteine residues of other proteins, the Taunton laboratory recently introduced a systemic approach towards the rational design of reversible covalent inhibitors [45]. This strategy was successfully employed to develop novel inhibitors of BTK and FGFR inhibitors with prolonged and tunable residence times *in vivo* [46].

Alternative approaches to PKI development

Next to inhibitors that directly decrease the enzymatic activity of PKs there are other, more indirect ways to interfere with kinase activity. Using CDKs as an example, to be fully active, these enzymes require i) synthesis of, and binding to, a regulatory cyclin subunit, ii) dissociation from inhibitor proteins, *e.g.* p16INK4A, and iii) phosphorylation by CDK7 and dephosphorylation by Cdc25. They can be inhibited by ubiquitin-dependent degradation of cyclins. Each one of these regulatory mechanisms is a potential drug target. To interfere with some of these processes would require the inhibition of protein-protein interactions, which is a difficult task, but as the example of the recently approved Bcl2 inhibitor venetoclax illustrates that it is not impossible [47]. Another promising avenue of drug discovery may be targeted protein degradation by so-called proteolysis-targeting chimeras (PROTACs) [48]. These bivalent heterobifunctional compounds recruit one of over 600 specific ubiquitin E3 ligases [49] to tag target proteins for subsequent degradation. The move from functional inhibition to destruction may also enable the targeting of proteins that have been deemed undruggable to the list of therapeutic targets. A different example of heterobivalent compounds is the mTOR inhibitor RapaLink-1, which consists of rapamycin linked to an ATP-competitive inhibitor [50]. This third-generation mTOR inhibitor effectively overcame acquired resistance to first- and second-generation inhibitors.

Recently, promising approaches have emerged by specifically targeting pseudokinases, which lack catalytic kinase activity but often function as scaffold proteins. Her3-dependent signaling in cancer cells was successfully inhibited with a heterobivalent compound, TX2-121-1, which contains a covalent inhibitor to selectively attack the ATP site of Her3 and a hydrophobic adamantane moiety to induce proteolytic degradation of thus modified protein [51]. A small molecule ligand of the JH2 pseudokinase domain of Tyk2, a member of the Janus tyrosine kinase family, was able to lock in a conformation that stabilizes an autoinhibitory interaction with the JH1 catalytic domain, thereby blocking Tyk2-mediated signaling important in autoimmunity [52]. A new class of pseudokinase antagonists was developed that stabilize a previously unrecognized inactive state of the scaffold protein KSR (kinase suppressor of Ras) [53]. In combination with MEK inhibitors, these compounds inhibited growth of Ras mutant cell lines, providing a new therapeutic strategy against Ras-driven cancers.

PKIs in diseases other than cancer

Adult cancers are typically characterized by aberrations in multiple signaling pathways, which has been and will continue to be a unique challenge for drug design and discovery. However, there are a number of other diseases that offer opportunities for PKI-based therapies. The Src family kinase inhibitor saracatinib undergoes clinical trials for

Alzheimer's disease, an example of efforts by the NIH to repurpose cancer drugs for the treatment of other diseases[54]. The rationale is that progression of the disease may be achievable by blocking the activity of Fyn kinase that has been shown to induce damage to brain synapses in mouse models of Alzheimer's [55]. Recent examples of PKIs in clinical trials for diseases other than cancer are the JAK inhibitors filgotinib and peficitinib for moderate-to-severe Crohn's disease [56] and rheumatoid arthritis [57], respectively, the ASK1 inhibitor selonsertib for diabetic kidney disease [58], and the ROCK inhibitor netarsudil for glaucoma and ocular hypertension [59].

Challenges in PKI drug discovery

Overcoming resistance

Despite the initial efficacy of PKIs, cancer cells frequently develop resistance by engaging multiple mechanisms (reviewed in [2,11]). In the case of acquired mutations in key residues within their catalytic domain, this can be overcome by second and third generation inhibitors. Examples of such inhibitors are the aforementioned osimertinib and the ALK inhibitor alectinib (approved in 2014), which is used in the treatment of crizotinib-refractory NSCLC [60]. The efficacy of inhibitors developed against cancers driven by ^{T790M}EGFR can be jeopardized by yet another acquired EGFR mutation, C797S. Recent work of Eck and coworkers describes the rational design of an allosteric EGFR inhibitor EA1045 that is effective against EGFR mutants but spares wild-type receptors in biochemical assays. To effectively inhibit EGF-driven cell proliferation required the combination with cetuximab, an antibody that prevents receptor dimerization [61]. The well-documented success of RAF inhibitors, for instance in metastatic melanoma, depends on the MAPK pathway to be virtually silent. Unfortunately, these inhibitors paradoxically stimulate MAPK signaling in cells harboring oncogenic RAS, leading to the rapid development of other skin tumors. Recently a research team from Plexxikon Inc. developed second-generation RAF inhibitors (PLX7904 and PLX8394) that were effective against cells with mutant ^{V600E}BRAF without activating the MAPK pathway [62]. Other "paradox-breaking" compounds that were recently reported are CCT196969 and CCT242161, pan-RAF inhibitors that also inhibit SRC [63]. These compounds were effective against melanoma cells and patient-derived mouse xenografts resistant to BRAF and BRAF/MEK inhibitors.

Conclusions

Nowadays, tumors are increasingly being characterized by molecular diagnostics, resulting in the creation of new tumor subtypes that go far beyond the anatomic and histologic categories. For instance, NSCLC can be subdivided into more than 10 distinct genotypes, with activating KRAS and EGFR mutations being the most frequent driver aberrations (15–25 and 10–35%, respectively) [64]. Thus pairing a drug targeting a certain kinase with patients that have acquired mutations or amplifications in this kinase are more successful. On the other hand, the fact that PKIs, like all targeted therapies, are tailor-made to particular, possibly small, patient populations, increases the cost of therapy significantly, an aspect that is often neglected in academic drug discovery campaigns. Of the 35 approved PKIs, nine compounds selectively inhibit five STKs, whereas the other 26 PKIs target just 11 receptor

tyrosine kinases (RTKs) and 5 non-receptor tyrosine kinase (NRTKs). Thus, only a very small subset of kinases are currently being exploited as a target for cancer therapy by FDA approved drugs, leaving a vast reservoir of yet poorly characterized kinases untapped. The second striking aspect is the absence, or near absence, of PKIs against several significant adult types of cancer, including pancreatic, prostate, colon and brain. The third is the fact that none of the approved drugs is purposefully aimed at pediatric tumors, which may be due to the expectation that such drugs would generate little revenue.

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Abbreviations

PKI	protein kinase inhibitor
RTK	Receptor tyrosine kinase
NRTK	Non-receptor tyrosine kinase
STK	Serine/threonine-specific kinase

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Figure 1.
FDA-approved small molecule kinase inhibitors.

Figure 2.
Chemical structures of representative kinase inhibitors.