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Journal

Nature Reviews Drug Discovery, 18(7)

ISSN

1474-1776

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Publication Date

2019-07-01

DOI

10.1038/s41573-019-0018-3

Peer reviewed



HHS Public Access

Author manuscript

Nat Rev Drug Discov. Author manuscript; available in PMC 2021 January 18.

Published in final edited form as:

Nat Rev Drug Discov. 2019 July ; 18(7): 501–526. doi:10.1038/s41573-019-0018-3.

Prospects for pharmacological targeting of pseudokinases

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Abstract

Pseudokinases are members of the protein kinase superfamily but signal primarily through noncatalytic mechanisms. Many pseudokinases contribute to the pathologies of human diseases, yet they remain largely unexplored as drug targets owing to challenges associated with modulation of their biological functions. Our understanding of the structure and physiological roles of pseudokinases has improved substantially over the past decade, revealing intriguing similarities between pseudokinases and their catalytically active counterparts. Pseudokinases often adopt conformations that are analogous to those seen in catalytically active kinases and, in some cases, can also bind metal cations and/or nucleotides. Several clinically approved kinase inhibitors have been shown to influence the noncatalytic functions of active kinases, providing hope that similar properties in pseudokinases could be pharmacologically regulated. In this Review, we discuss known roles of pseudokinases in disease, their unique structural features and the progress that has been made towards developing pseudokinase-directed therapeutics.

Introduction

Protein kinases constitute essential components of almost every signalling pathway. Most kinases are characterized by a highly conserved kinase domain fold that enables catalysis of phosphorylation, with specificity for serine, threonine or tyrosine residues in metazoans. Dysregulation of kinase function has been linked to numerous pathologies, most notably cancer, as well as neurological, immunological and metabolic diseases. In fact, the kinase domain is the most frequently observed domain in known oncogenes¹, and a multitude of genetic alterations in kinases, including changes in expression levels and mutations, have been described as driver events in malignant transformation. The therapeutic potential of kinases was evident shortly after their discovery, but the high degree of structural similarity in their active sites was initially thought to be prohibitive to the development of highly selective kinase inhibitors. Now, more than 30 small-molecule kinase inhibitors have been approved by the US Food and Drug Administration (FDA). These inhibitors largely consist

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Competing interests statement

The authors declare no competing interests.

of reversible ATP-competitive compounds and therefore directly target the ability of kinases to catalyse phosphorylation and counteract their hyperactivation in diseases².

Unbiased screens for somatic kinase mutations in cancer have identified a surprising number of mutations that are expected to impair kinase activity, rather than potentiate it, by replacing conserved residues in the active site^{3,4}. Although some of these inactivating mutations occur in kinases that act as tumour suppressors, such as LKB1 (also known as serine/threonine-protein kinase STK11), many others map to the kinase domains of bona fide oncogenic kinases that are typically hyperactivated in cancer, including BRAF, epidermal growth factor receptor (EGFR) and KIT. Indeed, kinase-inactivating mutations are the most common type of BRAF mutation in non-small-cell lung cancer (NSCLC)⁵, and recently, catalytically impaired BRAF mutants were shown to induce lung adenocarcinoma⁶. These mutations enhance a nonenzymatic function of BRAF, resulting in increased affinity for BRAF heterodimerization with CRAF, enhanced allosteric activation of CRAF and elevated mitogen-activated protein kinase (MAPK) pathway signalling⁷⁻⁹.

The ability of BRAF kinase-inactivating mutations to drive disease underscores the importance of the noncatalytic functions of kinases in signalling. In recent years, there has been a growing body of evidence demonstrating that many, if not all, kinases possess such functions in addition to their canonical role^{10,11,12}. Kinases can act as scaffolds, as observed for receptor-interacting serine/threonine-protein kinase 1 (RIPK1) and RIPK3, which are involved in assembling apoptotic signalling complexes¹³⁻¹⁵, or function as allosteric regulators of other enzymes, such as ERK2 (also known as MAPK1), which activates dual specificity protein phosphatase 6 (DUSP6; also known as MKP3)^{16,17}. Systematic analysis of the human genome has revealed that one-tenth of all protein kinases are predicted to be catalytically inactive and to therefore primarily signal through alternative mechanisms¹⁸. Members of this subset of the protein kinase superfamily are called pseudokinases and have inactivating mutations in critical catalytic motifs.

Pseudokinases, like their catalytically active counterparts, play pivotal roles in cellular signalling and are often dysregulated in a variety of diseases¹⁹⁻²¹. Compared with active kinases, however, pseudokinases pose a much greater challenge for drug design. The nucleotide-binding site is, by far, the most 'druggable' pocket in protein kinases owing to its evolved ability to interact with a small molecule. However, as discussed below, for many pseudokinases, it is unclear how molecules that compete with nucleotide binding could disrupt nonenzymatic functions. Moreover, a substantial number of pseudokinases do not bind a nucleotide, and several do not even seem to have a structurally defined pocket that could be accessible to ATP-competitive small molecules.

Although there have yet to be any clinically approved therapeutics that target pseudokinases, we are better equipped to meet this challenge than might be apparent at first glance. Structural and biochemical studies over the past decade have revealed that the regulation of noncatalytic kinase signalling carries substantial parallels to that of active kinases^{11,12}. The on and off states of catalytically competent kinases are characterized by defined architectures of the nucleotide-binding site and other structural elements within the kinase domain. Catalysis of phosphorylation relies on a regulated transition between these states.

Although it is unclear whether such transitions also occur in pseudokinases, several pseudokinases can adopt conformations that recapitulate features of either the on or off state of catalytically active protein kinases, and in many cases, these conformations are critical for their physiological roles. Therefore, molecules that could force pseudokinases to adopt another, non-functional conformation could be used for therapeutic targeting. Encouraged by examples of conformationally selective small molecules that modulate the noncatalytic functions of active kinases, which we discuss below, there is now growing interest in applying the principles of conformational selection to develop pharmacological agents that target pseudokinases. Here, we discuss these possibilities in light of our increasing knowledge of the structural mechanisms that underlie pseudokinase signalling.

Pseudokinases as disease targets

Pseudokinases are well conserved in evolution and comprise a notable proportion of the kinomes of not only humans and mice but also diverse species, including *Arabidopsis thaliana*, *Paramecium tetraurelia*, *Dictyostelium discoideum* and *Toxoplasma gondii*^{18,22–28} (Fig. 1). Although the functions of the vast majority of these pseudokinases remain poorly understood, studies conducted thus far highlight crucial roles for many of them in development, the immune response and metabolism. Consequently, mutations in pseudokinases and dysregulation of their expression have been linked to several developmental and morphological disorders, as well as a wide range of diseases, such as cancer, neurological disorders, metabolic disorders and autoimmune diseases^{19–21,29} (Table 1; see Supplementary Table 1 for a complete table with references).

HER3

One of the pseudokinases that emerged early as a potential therapeutic target is human epidermal growth factor receptor 3 (HER3; also known as ERBB3), a member of the EGFR (also known as HER or ERBB) family of receptor tyrosine kinases (RTKs). The known physiological functions of HER3 depend on its role as a co-receptor of catalytically active HER family members, including HER2 and EGFR, with which HER3 forms complexes upon growth factor binding. In these complexes, the HER3 pseudokinase domain allosterically activates its partner HER kinase by stabilizing it in the active conformation through the formation of an asymmetric kinase dimer^{30–32}. This allosteric functionality is encoded in all receptors in the EGFR family; HER3 has only this allosteric role and no catalytic role^{33,34}.

HER3 overexpression alone is insufficient to drive transformation³⁵, but its presence is required for the transformation of NIH3T3 cells by HER2³⁶. In HER2-overexpressing breast cancers and in EGFR-mutant-driven lung cancer and head and neck squamous cell carcinoma (SCCHN), HER3 promotes resistance to HER2 and EGFR inhibitors, respectively^{37–39}. HER3 is also associated with resistance to anti-oestrogen therapies in oestrogen receptor-positive breast cancer and with resistance to insulin-like growth factor 1 receptor (IGF1R) inhibitors in hepatocellular carcinomas^{40,41}. Drug-induced elevation of HER3 expression and phosphorylation are the primary mechanisms that contribute to this resistance (Fig. 2a). Somatic mutations that map to extracellular or intracellular regions of

HER3, including the pseudokinase domain, have also been identified in various types of cancer, including NSCLC, gastric cancer and colon cancer^{3,42–48}. The mutations in the extracellular domain might destabilize its autoinhibited conformation, thereby favouring HER3 dimerization with other members of the HER family. Several of these mutations promote ligand-independent cell survival and tumour growth both in vitro and in vivo in a HER2-dependent manner^{48,49} (Fig. 2a). The pseudokinase domain mutations map most frequently to the asymmetric kinase dimer interface and increase the affinity of the HER3–EGFR kinase heterodimer, which enhances the HER3-dependent allosteric activation of EGFR^{32,48}.

In addition to its oncogenic role in cancer, HER3 is also a potential target in neurological disorders. HER3 is essential for the growth and development of Schwann cells by functioning as a receptor for the growth factor neuregulin 1 (NRG1)⁵⁰. Mice homozygous for deletion of HER3 manifest severe defects in myelination, which lead to impaired motor and sensory neuron development; most animals die during early embryonic stages⁵⁰. Likely owing to the importance of HER3 for myelination, aberrant HER3 expression in humans is associated with a range of neurological diseases. Expression of HER3 is downregulated in the dorsolateral prefrontal cortex of patients with schizophrenia^{51–53}, and HER3 overexpression is observed in the Schwann cells of patients with Charcot–Marie–Tooth type 1 disease, one of the most frequently inherited neuropathies⁵¹. While the value of modulating the NRG1–HER3 axis in neurological disorders continues to be debated^{52,53}, its utility will ultimately be tested through the development of therapeutics that specifically and efficiently target HER3.

ROR1, ROR2, PTK7 and RYK

An unrelated group of RTKs, ROR1, ROR2, protein tyrosine kinase 7 (PTK7; also known as colon carcinoma kinase (CCK4)) and receptor-like tyrosine kinase (RYK), which are all categorized as pseudokinases, are co-receptors in WNT signalling pathways. Collectively, these pseudokinase receptors have been implicated in the pathology of several cancers and developmental disorders. ROR1 and ROR2 were originally identified as orphan RTKs that are highly expressed during embryonic development and have critical roles in skeletal and neural organogenesis⁵⁴. ROR1 and ROR2 have since been found to interact with the ligand WNT5A to mediate noncanonical WNT signalling through the planar cell polarity (PCP) pathway^{55,56}. Although ROR1 and ROR2 are not expressed in most normal adult tissues, the re-emergence of their expression through transcriptional upregulation is associated with various types of cancer. This makes RORs very attractive cancer targets, as they would potentially have few adverse effects in normal tissues. ROR1 is often overexpressed in haematological malignancies, including chronic lymphocytic leukaemia, in which WNT5A induces hetero-oligomerization of ROR1 and ROR2 to promote chemotaxis and proliferation⁵⁷. Upregulation of ROR1 is also observed in cancers that overexpress MET (also known as hepatocyte growth factor receptor), such as gastric carcinoma and NSCLC⁵⁸. Phosphorylation of the proline-rich domain and pseudokinase domain of ROR1 in these cells by MET and SRC, respectively, is important for MET-driven cell proliferation and invasion⁵⁹. Knockdown of ROR1 was found to reduce cell proliferation in multiple cancer cell lines and inhibited tumorigenesis in vivo in xenograft models^{58,60}.

ROR2 overexpression also promotes cell proliferation and migration in several types of cancer, such as osteosarcoma and renal cell carcinoma, and knockdown of ROR2 inhibits tumour growth both in vitro and in vivo^{61,62}. In some cancers, such as colon cancer and hepatocellular carcinoma, elevated ROR2 expression has the opposite effect and suppresses tumour growth^{63,64}. These contrasting roles of ROR2 may reflect its ability to both inhibit canonical β -catenin-dependent WNT signalling and activate noncanonical WNT signalling through the PCP pathway^{56,65}. Germline mutations in ROR2 are associated with autosomal dominant brachydactyly type B (BDB), a condition that is characterized by terminal deficiency of the fingers and toes, as well as an autosomal recessive form of Robinow syndrome (RRS), manifested by severe skeletal malformations and abnormal craniofacial features^{66–69}. RRS can be modelled using *Ror2*-deficient mice, suggesting that RRS-associated mutations lead to loss of function^{68–70}. BDB-associated mutations result in truncations of ROR2 before and after the pseudokinase domain, which are likely dominant-negative forms of the protein⁶⁶.

PTK7 interacts with WNT receptors, such as Frizzled and ROR2, as well as several WNT ligands, including WNT3A, WNT8 and WNT5A, to modulate both canonical and noncanonical WNT signalling^{71,72}. The PTK7 pseudokinase domain is essential for associating with β -catenin and interacts with the scaffold protein receptor of activated protein C kinase 1 (RACK1) to recruit Dishevelled to the plasma membrane^{73,74}. Although the mechanisms underlying its signalling remain poorly understood, PTK7 regulates several developmental and physiological processes that rely on cell migration, cell polarity and epidermal wound repair. Germline loss-of-function mutations in PTK7 are associated with idiopathic scoliosis and neural tube defects^{75,76}. The role of PTK7 in cancer remains somewhat mysterious. PTK7 is upregulated in some cancers, such as colon cancer, lung adenocarcinoma and breast cancer, but downregulation of PTK7 has been observed in ovarian carcinoma and metastatic melanoma^{77–81}. Experimental evidence suggests that inhibition of PTK7 signalling would likely be beneficial in cancers that overexpress PTK7. Knockdown of PTK7 reduces cell viability and increases apoptosis in lung cancer cells, inhibits tumour growth in lung adenocarcinoma xenografts and decreases motility and invasiveness in breast cancer cells^{78,79}.

RYK also contributes to both canonical and noncanonical WNT signalling, including the PCP and WNT–Ca²⁺ pathways. Like PTK7, RYK interacts with Frizzled and Dishevelled, as well as multiple WNT ligands, including WNT1, WNT3A and WNT5A⁸². At present, little is known regarding the mechanisms that underlie signalling by this pseudokinase receptor. RYK plays a pivotal role in the central nervous system by controlling axon guidance and neurogenesis^{83,84}. RYK also inhibits axon regeneration following spinal cord injuries in rats; therefore, blocking RYK-mediated signalling might improve recovery from such injuries⁸⁵. Overexpression of RYK has been associated with decreased overall survival and shorter progression-free survival in epithelial ovarian cancer⁸⁶, but the value of targeting RYK in cancer has yet to be explored.

JAKs

The Janus kinase (JAK) family of non-receptor tyrosine kinases, consisting of JAK1, JAK2, JAK3 and TYK2, contain a tandem kinase domain module in which the catalytically active tyrosine kinase domain (JAK homology 1, JH1) is preceded by a pseudokinase domain (JH2). The JH2 pseudokinase domain binds to the active JH1 kinase domain and stabilizes it in an inactive conformation^{87,88}. JAKs associate with the cytoplasmic portions of cytokine receptors and become activated by ligand binding to the receptors, resulting in the release of the autoinhibition imposed on the JH1 domain by the JH2 pseudokinase domain. Several mutations in the JH2 domains of JAK1, JAK2 or JAK3 result in constitutive activation of the JH1 kinase domain and are associated with myeloproliferative neoplasms, such as polycythaemia vera, and with acute myeloid leukaemia (AML)^{89–92}. Many of these mutations, such as V617F in the JH2 domain of JAK2, map near the JH1–JH2 interface, which likely disrupts the autoinhibitory lock that controls the activity of the active JH1 kinase domain^{93,94} (Fig. 2b). By contrast, some disease mutations in the JAK3 and TYK2 JH2 domains reduce activity of their JH1 domains^{95,96}, possibly by enhancing the autoinhibitory interaction between the pseudokinase and kinase domains. These JAK3 mutations have been linked to severe combined immunodeficiency⁹⁵, while the TYK2 mutations are associated with increased susceptibility to autoimmune disease⁹⁶. Thus, modulation of the extent of pseudokinase–kinase interactions in either direction in the JAKs could be beneficial in disease.

MLKL

As occurs in JAK proteins, the pseudokinase domain of mixed lineage kinase domain-like protein (MLKL) regulates the activity of an adjacent domain, denoted as the four-helix bundle (4HB) domain. MLKL is a key effector in necroptosis owing to its role in inducing plasma membrane permeabilization — a prerequisite for necroptotic cell death^{97–99}. Under normal conditions, the pseudokinase domain of MLKL stabilizes the 4HB domain in an inhibited conformation. Phosphorylation of the pseudokinase domain by RIPK3 triggers release of the 4HB domain, MLKL oligomerization and localization of MLKL to the cell membrane, where it induces permeabilization^{97–99}. The resulting loss of membrane integrity causes leakage of cellular contents, resulting in necroptotic cell death and, frequently, an inflammatory response that contributes to diseases, such as neurodegeneration, myocardial infarction and stroke, atherosclerosis, ischaemic–reperfusion injury and inflammatory bowel disease¹⁰⁰. Targeting necroptosis in these diseases is an attractive concept, and as the key effector of necroptosis, MLKL could serve as a novel target for the development of specific inhibitors that could avoid some of the undesirable effects of inhibitors developed to target RIPKs. Selective inhibitors of RIPK3 have been developed, but high concentrations of these compounds trigger apoptosis, and therefore they have limited therapeutic potential¹⁵. In addition to necroptosis, RIPK1 is involved in other processes, including nuclear factor- κ B signalling and apoptosis¹⁰¹, which will inadvertently be affected by RIPK1 inhibitors.

KSR

Kinase suppressor of RAS 1 (KSR1) and KSR2 pseudokinases in the RAF kinase family that function as allosteric regulators of active kinases, similar to HER3 and the JH2 domains

of JAKs. Both KSR1 and KSR2 promote activation of the MAPK pathway through interactions with RAF, MEK and ERK. An important step in RAF kinase activation is formation of a 'side-to-side' dimer in which one kinase allosterically stabilizes the active conformation in another¹⁰². This allosteric activator function is not dependent on catalytic activity, and hence KSR can activate RAF through heterodimerization, which results in MEK phosphorylation^{102,103}. KSR also binds directly to MEK, and recently, structural and biochemical studies revealed that this interaction further enhances the association between KSR and BRAF¹⁰⁴.

Owing to its positive effect on MAPK signalling, KSR has become a therapeutic target of interest in the treatment of RAS-driven cancers. KSR1 is necessary for tumorigenesis in a mouse model of skin cancer driven by the oncogenic RAS G12V mutation, and deletion of KSR1 in mouse embryonic fibroblasts prevents RAS G12V-induced transformation^{105,106}. KSR1-null mice develop normally, which indicates that inhibition of KSR1 in adults with cancer might have low toxicity^{105,107}. By contrast, KSR2-null mice develop diabetes and hypertension^{108,109}, and mutations in KSR2, including several in the pseudokinase domain, are associated with insulin resistance and obesity¹¹⁰. These mutations impair the ability of KSR2 to stimulate MAPK signalling and are predicted to disrupt interactions with RAF and MEK (Fig. 2c). Hence, inhibition of KSR might disrupt glucose homeostasis^{108,109}, unless inhibitors specific to KSR1 are developed.

FAM20A

FAM20A is another pseudokinase that modulates the activity of a closely related active kinase, FAM20C. FAM20A and FAM20C are members of a recently characterized family of atypical Golgi-localized kinases that undergo secretion to the extracellular space^{111,112}. Both of these proteins have critical roles in biomineralization and enamel development. FAM20A allosterically activates FAM20C and promotes its secretion^{112,113}. These functions of FAM20A are abrogated by mutations associated with the biomineralization disorder amelogenesis imperfecta^{112,113,114} (Fig. 2d). Mutations in FAM20C result in Raine syndrome, a rare disorder characterized by severe and often lethal osteosclerotic bone dysplasia¹¹⁵. FAM20C was recently found to be responsible for phosphorylating the majority of the secreted phosphoproteome — a function disrupted in Raine syndrome by mutations that impair FAM20C catalytic activity^{112,116}. Strikingly, FAM20A potentiates the activity of FAM20C Raine syndrome mutants *in vitro*¹¹², suggesting that enhancing the interaction between FAM20A and FAM20C could be beneficial in the treatment of Raine syndrome.

Tribbles

The Tribbles family of pseudokinases (TRIB1, TRIB2 and TRIB3) stands out among pseudokinases because these proteins have been described as scaffolds for diverse signalling proteins in seemingly unrelated signalling pathways, such ubiquitylation of transcription factors or regulation of kinases in the MAPK and AKT signalling pathways. Consequently, TRIBs have been implicated in a variety of diseases, including several types of cancer and metabolic disease.

One of the best understood functions of TRIBs is their role in regulation of ubiquitylation by the E3 ubiquitin ligase COP1. The C-terminal tails of TRIBs contain a highly conserved motif that recruits COP1, which then ubiquitylates substrates that interact with TRIB, such as the transcription factor CCAAT enhancer-binding protein- α (C/EBP α) and the metabolic enzyme acetyl-CoA carboxylase^{117–120}. Evolutionary analysis of TRIB homologues, compared with the rest of the kinome, revealed that the C-terminal COP1-binding motif is a defining feature of this family of kinases¹²¹. Overexpression of TRIB1 or TRIB2 induces leukaemogenesis in mice through depletion of C/EBP α in a COP1-dependent manner, implicating upregulation of COP1 function by TRIB1 and TRIB2 in AML^{117,119,122} (Fig. 2e). In addition, likely owing to the role of C/EBP α in lipogenesis, genome-wide association studies have identified variants at the TRIB1 locus that significantly associate with cardiovascular disease and plasma levels of triglycerides, cholesterol, low-density lipoprotein and high-density lipoprotein¹²³.

The mechanisms by which TRIB family pseudokinases modulate kinases within the MAPK and AKT pathways are much less understood. TRIB1, TRIB2 and TRIB3 interact with MEK through a conserved motif in the C-terminal lobes (C-lobes) of their pseudokinase domains, and this interaction increases ERK phosphorylation¹²⁴. TRIB-mediated activation of the MAPK pathway has been implicated in both leukaemia and breast cancer^{124,125}. In the AKT pathway, different TRIBs seem to play opposing roles. TRIB3 promotes insulin resistance by binding to and inhibiting AKT, which leads to increased apoptosis of pancreatic β -cells and reduced insulin secretion^{126,127}. Elevated levels of TRIB3 and the resulting inhibition of AKT are also associated with neuronal cell death in Parkinson disease through the promotion of apoptosis^{128,129}. By contrast, TRIB2 enhances AKT signalling, and overexpression of TRIB2 promotes drug resistance in cancer cells¹³⁰. Collectively, these findings indicate that there is still much to learn about the mechanisms of TRIB signalling and that inhibition of TRIB function would potentially be therapeutically beneficial in a variety of diseases.

TRRAP

Another pseudokinase that functions as a scaffold is transformation/transcription domain-associated protein (TRRAP), a member of the phosphatidylinositol 3-kinase-related kinase (PIKK) family. TRRAP plays a critical role in tumorigenesis, as it is essential for transformation driven by the oncogenic transcription factors, MYC and E2F, as well as by the adenovirus E1A oncoprotein^{131,132}. TRRAP serves as a component of several histone acetyltransferase (HAT) complexes, including the STAGA, TFTC and TIP60 complexes^{133–135}. Through its interactions with oncoproteins, such as MYC and E1A, TRRAP recruits HAT complexes to chromatin to promote histone acetylation and stimulate transcription^{136–138}. The pseudokinase domain of TRRAP is required for assembly of functional HAT complexes and for MYC-driven oncogenic transformation¹³⁹. Although the TRRAP pseudokinase domain does not bind directly to MYC, it does interact with HATs¹³⁹. Consequently, small molecules targeting TRRAP could potentially be used therapeutically to target MYC-driven cancers by preventing HAT recruitment to sites of MYC-dependent transcription.

Structural features of pseudokinases

The crystal structures of pseudokinase domains that have been resolved to date show that pseudokinases have the same overall kinase domain fold as catalytically active kinases^{11,12}. This highly conserved fold is composed of two lobes, the amino-terminal lobe (N-lobe) and the C-lobe, which are connected via a flexible hinge region (Fig. 3). In active kinases, the relative motions of these lobes during catalysis are associated with conformational transitions in the active site, located between the N-lobes and C-lobes and composed from structural elements provided by both lobes. These motions serve to accommodate nucleotide and substrate binding, ATP hydrolysis, phosphotransfer and the eventual release of ADP and the phosphorylated substrate^{140,141}. The conformational states that correspond to the different steps of catalysis have been captured in crystal structures of active kinases and, interestingly, are emulated by some pseudokinases in their crystal structures.

By definition, pseudokinases lack one or more key conserved catalytic residues: the lysine within the VAIK (Val-Ala-Ile-Lys) motif, the aspartate within the HRD (His-Arg-Asp) motif and the aspartate within the DFG (Asp-Phe-Gly) motif¹⁸. The VAIK motif is located in the β 3 strand of the N-lobe, which positions the catalytic lysine to coordinate the α and β phosphates of ATP. Proper coordination of ATP is dependent on salt bridge formation between the catalytic lysine and a conserved glutamate residue in helix α C, which is rotated towards the active site in the kinase active state (Fig. 3a). This salt bridge is disrupted in an inactive conformation of the kinase, referred to as the SRC or cyclin-dependent kinase (SRC/CDK)-like inactive conformation, in which helix α C is rotated away from the active site¹⁴² (Fig. 3b). Mutation of the β 3 lysine is often used as an inactivating mutation to generate a 'kinase-dead' mutant. Alteration of this lysine is observed in some pseudokinases, including KSR and STE20-related adaptor- α (STRAD α). Other pseudokinases compromise the salt bridge through mutation of the helix α C glutamate, which is sometimes accompanied by even more pronounced structural changes in helix α C. In HER3, the canonical glutamate is replaced by a histidine, and helix α C is partially unwound^{30,143}. A central residue in the unstructured region, Phe734, which is absent in active HER kinases, forms hydrophobic interactions that stabilize the activation loop and helix α C in the SRC/CDK-like inactive conformation (Fig. 3d). Similar to HER3, helix α C in TRIB1 lacks the conserved glutamate and is shorter than it is in most protein kinases. In addition, it adopts an atypical kink that interferes with the canonical structure of the nucleotide-binding pocket and likely contributes to the inability of TRIB1 to bind nucleotides¹⁴³ (Fig. 3e). Pragmin (also known as SGK223 or PRAG1) and PEAK1 (also known as SGK269) also lack the conserved glutamate residue, and their structures hint at either disorder or a high degree of conformational dynamics within the helix α C region. The N-terminal region of helix α C in PEAK1 has only two helical turns visible in the structure¹⁴⁴, while in structures of Pragmin, helix α C is entirely disordered^{145,146} (Fig. 3f).

The HRD motif located in the catalytic loop is another key motif required for catalysis. The aspartate residue in this motif serves as the catalytic base during ATP hydrolysis, and thus its mutation renders kinases catalytically inactive. Several pseudokinases carry HRD mutations or are missing this motif entirely, including HER3, integrin-linked protein kinase (ILK) and MLKL (Table 2). Other sequence alterations in pseudokinases include substitutions in the

glycine-rich loop located in the N-lobe. The glycine-rich loop usually conforms to the consensus sequence GXGXXG in active kinases. The absence of side chains in the glycine residues allows for close contact of the glycine-rich loop with the adenosine ring of ATP, which enables nucleotide binding and proper positioning of ATP for catalysis. In pseudokinases, the glycines are often replaced by larger amino acids, frequently negatively charged, that interfere with ATP binding. This is exemplified by VRK3, in which Asp175 and Gln177 within the glycine-rich loop protrude into the putative ATP-binding pocket¹⁴⁷. In TRIB1, Glu101 and Glu103 in the glycine-rich loop are predicted to interfere with ATP binding, and the loop is also shorter than usual, which makes the ATP-binding pocket relatively shallow¹⁴³ (Fig. 3e).

Conformational transitions of the activation loop, which adopts a tethered conformation in the inactive state and extends during activation, are necessary for catalysis and substrate binding in active kinases (Fig. 3a–c). This extended conformation is typically promoted by phosphorylation within the activation loop and is sometimes stabilized by an interaction with a binding partner¹⁴⁰. Conformational changes within the activation loop are sometimes accompanied by the motions of the N-terminally located DFG motif. In the active ‘DFG-in’ conformation, the aspartate points into the active site and coordinates a Mg²⁺ ion that interacts with the β -phosphate and γ -phosphate of ATP. In the inactive, ‘DFG-out’ state, the aspartate rotates $\sim 180^\circ$ away from the active site, and its position in the ATP-binding site is occupied by the neighbouring phenylalanine (Fig. 3c). The DFG-in versus DFG-out states of kinases often control the interactions of active kinases with their binding partners. For example, ERK2 loses the ability to serve as an allosteric activator of the phosphatase MKP3 when stabilized in the DFG-out conformation by a type II inhibitor. By contrast, locking ERK2 in the DFG-in state using a type I inhibitor enhances MKP3 activation¹⁴⁸.

Many pseudokinases carry mutations in their DFG motifs or lack this motif entirely (Fig. 2). Those that retain an intact DFG motif often have other alterations that likely prevent the canonical conformational changes observed in active kinases, such as the unusually short activation loops in RNase L and TYK2^{93,149,150}. Nevertheless, the emerging theme from structural studies on pseudokinases is that, even when they do not retain canonical motifs like the DFG motif, pseudokinases seem to adopt conformations that resemble the DFG-in, DFG-out or SRC/CDK-like inactive states. These states are achieved in pseudokinases through novel interactions that accommodate their divergent sequences. HER3, for example, has been repeatedly crystallized in the SRC/CDK-like inactive conformation in which it is stabilized through hydrophobic interactions that are missing in other HER kinases^{30,32,151}. STRAD α adopts a conformation that resembles the active conformation seen in active kinases^{152,153}, which is stabilized in a unique fashion by a salt bridge between Asp232 in the activation loop of STRAD α and Arg194 in the catalytic loop (Fig. 3g). ROR2, however, seems to adopt a conformation that is a hybrid of the DFG-out and SRC/CDK-like inactive states. In ROR2, the phenylalanine in the DFG motif is replaced by a leucine residue that points into the pseudoactive site, mimicking a DFG-out-like state¹⁵⁴. This DLG motif in ROR2 is positioned 4.0–4.5 Å away from the usual location of the DFG motif in active kinases, making room for the activation loop to fold into the active site and subsequently push helix α C away, in a manner reminiscent of the SRC/CDK-like inactive state¹⁵⁴ (Fig. 3h). Tyr555 in the hinge region of ROR2 directly inserts into the nucleotide-binding pocket

and structurally occludes ATP binding in this unique conformational state of the ROR2 pseudokinase (Fig. 3h). Thus, structural features of pseudokinase domains, while mirroring conformational states of active kinases, also seem to be unique and are likely intimately coupled to pseudokinase functions.

Conformational transitions in pseudokinases

Although we know much less about the ability of pseudokinases to toggle between different conformational states and how such changes regulate their functions, structural studies point towards the potential for such mechanisms. Human and murine MLKL adopt strikingly different conformations in crystal structures, suggesting the potential regulation of MLKL function through conformational changes in the activation loop and helix α C. The human MLKL pseudokinase domain was crystallized in a canonical 'active' conformation in which the β 3 lysine (Lys230) forms a salt bridge with Glu250 in helix α C^{155–157}. In structures of murine MLKL, however, the β 3 lysine (Lys219) interacts with Gln343 in the activation loop, which forms an unusual helix that takes the position in the kinase domain typically occupied by helix α C^{97,155} (Fig. 3i). Mutations that disrupt the noncanonical interaction between the β 3 lysine and the activation loop helix result in constitutive activation of MLKL-mediated necroptosis⁹⁷. Thus, the MLKL pseudokinase domain could potentially toggle between these two different conformational states to control necroptosis.

More direct evidence that conformational transitions underlie pseudokinase signalling has been recently provided by structures of TRIB1. A crystal structure of the TRIB1 pseudokinase domain with its C-terminal tail revealed that the tail binds to a pocket formed by helix α C in the N-lobe of the pseudokinase domain in an interaction reminiscent of the autoinhibitory binding of the C-terminal tails of AGC family kinases to the 3-phosphoinositide-dependent protein kinase 1 (PDK1)-interacting fragment (PIF) pocket^{143,158} (Fig. 3f). This intramolecular interaction effectively buries the COP1-binding motif, located in the TRIB1 C-terminal tail, such that, in this conformation, TRIB1 would be unable to bind COP1. More recently, binding of the COP1 substrate C/EBP α to the C-lobe of TRIB1 was found to induce long-range conformational changes in the TRIB1 pseudokinase domain, including ordering of the activation loop into a DFG-in-like state and displacement of the C-terminal tail from the N-lobe¹²⁰. Hence, binding of C/EBP α to TRIB1 has an allosteric effect and exerts positive feedback on COP1-dependent C/EBP α ubiquitylation by increasing the accessibility of the TRIB1 C-terminal tail for binding to COP1. These studies present a compelling example of how the conformational dynamics of a pseudokinase domain can be coupled to its function.

Classes of pseudokinases

Pseudokinases diverge from the canonical structural features of catalytically active kinases to different extents. Numerous pseudokinases retain the capacity to interact with nucleotides and divalent cations, and within this group, a few pseudokinases even have measurable kinase activity *in vitro*. A comprehensive analysis of these features across a broad spectrum of pseudokinases led to their categorization into four distinct classes. Class 1 pseudokinases do not bind nucleotides or cations; class 2 pseudokinases bind nucleotides in the absence of

cations; class 3 pseudokinases bind cations but not nucleotides; and class 4 pseudokinases bind nucleotides and cations¹⁵⁹ (Fig. 4; Table 2).

Class 1 — pseudokinases that do not bind nucleotides or cations

A substantial number of pseudokinases, including TRIB1, VRK3, ROR2, Pragmin and the bacterial pseudokinase MviN, lack the ability to bind traditional ligands, such as nucleotides and metal cations, in their pseudoactive sites¹⁵⁹. Crystal structures of several of these pseudokinases offer an explanation for this deficiency by revealing highly distorted nucleotide-binding sites, often occluded by hydrophobic residues^{143,145–147,154,160} (Figs 3,4). Effectively, such pseudokinases do not have the canonical druggable pocket present in active kinases, which constitutes a major challenge for targeting using small-molecule therapeutics (Fig. 4b). However, these divergent pseudoactive sites could potentially accommodate nonconventional ligands, either physiological or experimentally developed. As discussed in more detail below, close homologues of several class 1 pseudokinases, such as TRIB2 and PEAK1, retain the ability to bind canonical kinase ligands even though their pseudoactive sites are similarly distorted.

Class 2 — pseudokinases that bind nucleotides in the absence of cations

Divalent metal binding, most typically of Mg^{2+} , neutralizes the net negative charge of the nucleotide and enables nucleotide binding to the kinase active site; divalent metals are also essential for ATP coordination and the chemistry of phosphotransfer¹⁶¹. However, several pseudokinases, such as STRAD α , MLKL, FAM20A, CASK and TRIB2, retain the ability to bind to ATP even in the absence of cations^{97,152,162–164}. In fact, the presence of Mg^{2+} inhibits nucleotide binding to some of these pseudokinases^{97,162,164}. With the exception of FAM20A, most of the pseudokinases within this class lack the canonical metal-coordinating aspartate residue found in the DFG motifs of active kinases (Table 2). In the remarkable case of STRAD α , the pseudoactive site lacks the β 3 lysine, DFG motif and the catalytic aspartate in the HRD motif but still binds ATP in an orientation similar to that observed for active kinases. The phosphates of ATP are coordinated by basic residues that occupy the space in the STRAD α pseudoactive site where Mg^{2+} is coordinated by the DFG aspartate in active kinases¹⁵² (Fig. 3g). In FAM20A, although present, the DFG motif is not involved in ATP binding, perhaps owing to the lack of metal coordination. In a manner somewhat similar to STRAD α , ATP is coordinated by basic residues in FAM20A, but ATP binds to FAM20A in a unique orientation in which it is inverted relative to the position the nucleotide adopts in canonical kinases¹⁶⁴.

Unexpectedly, CASK and TRIB2 have been reported to catalyse phosphorylation *in vitro*, but only in the presence of a metal chelator, such as EDTA^{162,163}. Although there is no crystal structure of TRIB2 to date, the crystal structure of the CASK pseudokinase domain bound to a nonhydrolysable ATP analogue, AMP-PNP, does not reveal an obvious mechanism for ATP hydrolysis. The β -phosphate and γ -phosphate of the nucleotide, which are typically coordinated by Mg^{2+} in structures of canonical kinases, are disordered in the CASK structure¹⁶³. Owing to the high cellular concentrations of Mg^{2+} , it also remains unclear how CASK and TRIB2 would be able to efficiently catalyse phosphorylation in cells. As CASK plays a key role in synaptic function, it has been proposed that its catalytic

activity could be regulated by the changes in cellular Mg^{2+} and Ca^{2+} concentrations associated with synaptic activity¹⁶³.

Class 3 — pseudokinases that bind cations but not nucleotides

One surprising outcome of the analysis performed by Murphy and colleagues was the identification of a distinct class of pseudokinases that bind divalent cations but not nucleotides or ATP-competitive small molecules¹⁵⁹. This small class of pseudokinases is currently composed of the Toxoplasma protein rhoptyr protein 2 (ROP2) and PEAK1, a close homologue of Pragmin. Crystal structures of the pseudokinase domains of PEAK1 and ROP2 reveal that both possess highly occluded pseudoactive sites that seem structurally incompatible with nucleotide binding^{144,165} (Fig. 4d). Thus far, divalent cations have not been resolved in any of the crystal structures of either PEAK1 or ROP2, which leaves their binding site undefined^{144,165,166}. As neither of these pseudokinases possesses an intact DFG motif (Table 2), it is likely that these binding sites will not resemble those of canonical kinases. It remains unclear whether cations bind to PEAK1 and ROP2 in a physiological setting and what role metal ion binding plays in the function of these pseudokinases. A documented example of MAPK kinase signalling regulation by copper binding sets an intriguing precedent for the unconventional allosteric role of metal ligands in the regulation of protein kinases¹⁶⁷.

Class 4 — pseudokinases that bind nucleotides and cations

Class 4 pseudokinases retain the ability to bind both nucleotides and divalent cations and include HER3, KSR, RNase L and the JH2 pseudokinase domains of the JAKs (Table 2). Typically, these pseudokinases have an intact DFG aspartate, but there are exceptions, such as the interleukin-1 receptor-associated kinase-like 2 (IRAK2) and the plant pseudokinase BSK8 (Table 2). Many of the pseudokinases in this class, including HER3, the JH2 domain of JAK2 and KSR, have measurable kinase activity, typically a few orders of magnitude lower than that of catalytically active kinases. For example, HER3 does not have measurable activity towards a generic tyrosine substrate peptide *in vitro*³⁰, but its autophosphorylation activity towards its C-terminal tail can be detected *in vitro*, albeit the activity is ~1,000-fold lower than that of EGFR towards its respective C-terminal tail¹⁵¹. HER3 pseudokinase domain mutations that further reduce this activity do not have a measurable effect on HER3-mediated signalling in cells¹⁶⁸.

The JH2 pseudokinase domain of JAK2 was demonstrated to have weak autophosphorylation activity *in vitro*¹⁶⁹. Engineered mutations that disrupt ATP binding to the JH2 domain have only minor effects on wild-type JAK2 signalling, but they prevent the hyperactivation that occurs in the pathogenic JAK2-V617F mutant¹⁷⁰. Mutation of the putative JH2 autophosphorylation sites has no effect on the hyperactivity of the JAK2-V617F mutant¹⁷⁰, indicating that the ability of the JH2 domain to bind ATP — not JH2 catalytic activity — contributes to the pathogenic signalling by JAK2-V617F. As suggested by molecular dynamics simulations, ATP binding might play a structural role in stabilizing helix αC in the JH2 domain, which is intrinsically more disordered in disease-associated JAK2 mutants^{170,171}. Although no activity has been associated with the highly homologous JH2 domain of JAK1, hyperactivating disease-associated mutations in JAK1 JH2 that are

analogous to those found in JAK2 JH2, such as JAK1-V658F, also seem to rely on ATP binding to the JH2 domain for pathological signalling¹⁷⁰.

KSR1 and KSR2 were reported to become catalytically activated through heterodimerization with BRAF or CRAF, resulting in phosphorylation of MEK^{103,172}. This phenomenon was detected upon treatment with the RAF inhibitors PLX4720 or GDC-0879 and led to the hypothesis that KSR kinase activity might be involved in paradoxical activation of the MAPK pathway^{103,172}. More recently, however, mutations in the KSR1 active site that were expected to ablate KSR activity had no impact on MEK phosphorylation¹⁰⁴. By contrast, inactivating mutations in BRAF reduced MEK phosphorylation, indicating that the kinase activity of BRAF, and not that of KSR, is required for MEK phosphorylation by the BRAF–KSR complex¹⁰⁴. Consequently, the allosteric function of KSR, rather than its potential catalytic activity, appears to be more important for its role in MAPK signalling. Thus, as with the class 2 pseudokinases, CASK and TRIB2, the physiological relevance of the catalytic activity associated with class 4 pseudokinases remains unclear.

Targeting the nucleotide-binding site

Lessons from paradoxical effects of inhibitors targeting active kinases

Occupancy of the nucleotide-binding pocket in catalytically active protein kinases can be coupled to conformational changes in distal parts of the kinase domain and, in this way, allosterically regulate interactions of kinases with their binding partners^{11,173–175}. The importance of these long-range interactions is underscored by numerous studies that revealed paradoxical effects of ATP-competitive inhibitors on their target kinases. A prototypical example is provided by BRAF inhibitors, including both type I and type II inhibitors, such as GDC-0879 and AZ-628, respectively, that counterintuitively cause activation of the MAPK pathway at subsaturating concentrations¹⁷⁶. These inhibitors stabilize helix α C of BRAF in the ‘in’ position and promote dimerization with CRAF, resulting in its allosteric activation and enhanced binding of BRAF to RAS-GTP^{177–181}. BRAF dimerization also undermines the efficiency of another class of RAF inhibitors, including vemurafenib and dabrafenib, which stabilize helix α C in the ‘out’ position. The BRAF dimerization interface is not sterically compatible with both protomers adopting an α C-out conformation. Therefore, only the inhibitor-bound protomer is in an α C-out state, and the other protomer is forced to adopt an α C-in conformation that is competent to bind ATP and activate MAPK signalling¹⁸⁰. Understanding the structural basis for the activating effect of these inhibitors enabled design of next-generation BRAF inhibitors that stabilize the kinase in a conformation that is not compatible with BRAF–CRAF dimerization or interaction with RAS-GTP^{180,182,183}. Another strategy to avoid paradoxical activation utilizes pan-RAF inhibitors that target both BRAF and CRAF to prevent the CRAF-mediated phosphorylation of MEK that is induced by BRAF-selective inhibitors¹⁸⁴.

A somewhat similar mechanism appears to regulate PKR-like ER kinase (PERK; also known as EIF2 α K3), a transmembrane kinase sensor involved in the unfolded protein response. Subsaturating concentrations of an ATP-competitive inhibitor of the PERK kinase domain induce dimerization and activation of PERK kinase activity¹⁸⁵. This is thought to occur through a mechanism reminiscent of the paradoxical activation of BRAF in which the

inhibitor stabilizes the kinase domain of PERK in a dimer where only the active site of one protomer is occupied by the compound. This inhibitor-bound protomer could then allosterically activate an apo protomer that is still capable of binding ATP.

Another example of successful pharmacological modulation of noncatalytic kinase signalling is that of IRE1, another transmembrane regulator of the unfolded protein response. IRE1 oligomerization induced by binding of misfolded proteins on the luminal side of the endoplasmic reticulum results in activation of the kinase on the cytosolic side. Activation and autophosphorylation of the kinase domain then promote RNase domain activation. The same effect can be achieved by binding of a type I inhibitor to the IRE1 kinase domain^{186,187}. By stabilizing the kinase domain in an active DFG-in conformation, type I inhibitors promote IRE1 oligomerization, which is the critical determinant of RNase domain activation^{186–188}. By contrast, compounds that lock the IRE1 kinase domain in an inactive DFG-out conformation prevent both kinase oligomerization and activation of the RNase domain^{188–190}.

Conformation-selective inhibitors of Aurora kinase A were also found to modulate the extent of its protein–protein interactions. Aurora kinase A is often overexpressed in cancer, and one of its therapeutically important functions is to stabilize the oncogenic transcription factor N-MYC through a direct interaction¹⁹¹. This function of Aurora kinase A is independent of its kinase activity. Type I kinase inhibitors, such as hesperadin, do not interfere with N-MYC binding to Aurora kinase A. By contrast, another class of Aurora kinase A inhibitors, including MLN8054, MLN8037 and CD532, successfully disrupts binding of N-MYC by stabilizing an unusual conformation of the DFG motif that shifts helix α C away from the active site of Aurora kinase A^{192,193}.

Most recently, the ATP-competitive HER2 inhibitor, lapatinib, was found to cooperate at low concentrations with the growth factor NRG1, a HER3 ligand, to promote the proliferation of HER2+ breast cancer cells¹⁹⁴. Lapatinib stabilizes a SRC/CDK-like inactive conformation of the HER2 kinase domain that is incompatible with heterodimerization with HER3 in the canonical asymmetric dimer mode in which HER2 is allosterically activated by HER3^{32,33,195,196}. Instead, lapatinib-bound HER2 was shown to engage in different HER2–HER3 heterodimers driven by symmetrical ‘head-to-head’ N-lobe interactions, which were previously observed in crystal structures of the HER3 pseudokinase domain^{30,194} and the inactive HER4 kinase domain¹⁹⁶. These symmetrical heterodimers composed of inhibited HER2 and the pseudokinase HER3 both have their allosteric activator interfaces available for the formation of canonical asymmetric dimers with other HER receptors. Indeed, lapatinib was shown to promote higher-order HER2 and HER3 receptor oligomers that are thought to serve as nucleation points for the recruitment of other HER receptors and their activation through asymmetric kinase dimerization¹⁹⁴. These larger clusters of receptors could explain how lapatinib promotes downstream HER2–HER3 signalling, much as EGFR clustering has been functionally linked to EGFR signalling^{197,198}.

Known ATP-competitive modulators of pseudokinases

Collectively, what we have learned from these studies of active kinases is that ATP-competitive small molecules can be used to manipulate the noncatalytic functions of kinases

by stabilizing conformations that disrupt existing interactions or by promoting novel interactions that result in a modified signalling output. Multiple examples support this approach as a viable strategy for therapeutic targeting of pseudokinases that have an accessible ATP-binding pocket²⁹ (Table 3).

The allosteric function of KSR2 can be modulated by an ATP-competitive small molecule, APS-2-79, which stabilizes a conformation of KSR2 that is incompatible with its interaction with RAF. This compound reduces RAF-mediated phosphorylation of MEK by promoting the interaction of KSR2 with MEK, which results in occlusion of the Ser218 and Ser222 phosphorylation sites on MEK¹⁹⁹ (Fig. 5a). By contrast, the type I inhibitor ASC-24, which stabilizes a more ‘active-like’ conformation of the KSR2 pseudokinase domain, does not interfere with the ability of KSR2 to heterodimerize with RAF and RAF-mediated phosphorylation of MEK^{172,199}.

In HER3, occupancy of the nucleotide-binding pocket seems to play a structural role that enables it to heterodimerize with HER2 through both asymmetric and symmetric dimers¹⁹⁴. Mutations in HER3 that prevent ATP binding abrogate NRG-induced phosphorylation of HER3 by HER2, as well as lapatinib-induced symmetric heterodimerization with HER2. By contrast, the ATP-competitive inhibitor bosutinib, which binds to HER3 with nanomolar affinity and stabilizes the same conformation of the HER3 pseudokinase domain as ATP does, promotes HER2–HER3 heterodimerization and ligand-independent proliferation of HER2+ breast cancer cells¹⁹⁴. These findings suggest that molecules that occupy the nucleotide-binding pocket of HER3 in a similar fashion to ATP promote its heterodimerization with other HER kinases and downstream signalling.

Compounds that bind to the JH2 pseudokinase domain of TYK2, but not the JH1 kinase domain, were identified in a chemogenomic screen for kinase inhibitors that antagonize interleukin-23 receptor (IL-23R) signalling. These molecules inhibited TYK2 autophosphorylation, as well as TYK2-mediated phosphorylation of signal transducer and activator of transcription 3 (STAT3) in cellular assays, but had no effect on the enzymatic activity of the purified JH1 kinase domain *in vitro*²⁰⁰. This effect could be achieved if the compounds prevent activation of the JH1 domain indirectly through binding to the JH2 pseudokinase domain²⁰⁰. In the crystal structure of the JH2 pseudokinase domain bound to one of these compounds, BMS-066, the JH2 pseudokinase domain adopts a SRC/CDK-like inactive conformation, similar to that observed in the structure of the autoinhibited form of the pseudokinase–kinase module of TYK2^{93,200}. Thus, the inhibitory effect of compounds, such as BMS-066, was proposed to result from stabilization of an autoinhibited conformation of TYK2. Further optimization of the top hits from the screen yielded inhibitors with even greater potency, metabolic stability and selectivity for the TYK2 JH2 domain²⁰¹. In a phase II clinical trial, BMS-986¹⁶⁵, which binds selectively to the TYK2 JH2 domain and potently inhibits activation of the JH1 kinase domain, was found to substantially enhance skin clearance in patients with moderate-to-severe psoriasis²⁰². Phase III studies evaluating BMS-986¹⁶⁵ in psoriasis are currently enrolling ([NCT03611751](#) and [NCT03624127](#)), while phase II studies of this compound for treatment of Crohn’s disease and systemic lupus erythematosus are ongoing ([NCT03599622](#) and [NCT03252587](#)).

Several ATP-competitive small molecules, including the pan-CDK and Aurora inhibitor JNJ-7706621 and the Aurora, JAK2 and JAK3 inhibitor AT-9283, were found to bind to the JH2 domain of JAK2^{203,204}. In co-crystal structures of the JAK2 JH2 pseudokinase domain in complex with these compounds, the JH2 domain adopts the same conformation as TYK2 JH2 bound to BMS-066, suggesting that these compounds could potentially inhibit JAK2 signalling by stabilizing an autoinhibitory interaction between the JH1 and JH2 domains^{200,203}. Further studies are needed to increase the selectivity of these compounds and to determine whether they can indeed modulate JAK2 signalling.

Following the mode of its active counterpart, IRE1, in which nucleotide binding to the kinase domain activates the adjacent RNase domain through oligomerization, the pseudokinase domain of RNase L requires nucleotide binding to allosterically activate its RNase domain^{150,205}. ATP, ADP and, to a slightly lesser extent, AMP-PNP exert this activating effect through binding to the RNase L pseudokinase domain¹⁵⁰. Structural studies demonstrate that these nucleotides stabilize the pseudokinase domain in a conformation reminiscent of the active DFG-in state of catalytically active kinases, indicating that, as observed for IRE1, this conformation of the pseudokinase domain is important for RNase activation in RNase L^{149,150}. Several compounds, including sunitinib, an ATP-competitive inhibitor of multiple RTKs, as well as flavonols, such as quercetin, were found to inhibit RNase L activity and dimerization²⁰⁶. Addition of an excess of ADP overcomes the inhibitory effect of these compounds, indicating that both sunitinib and the flavonols act through the ATP-binding pocket of the RNase L pseudokinase domain, likely by stabilizing a conformation that is incompatible with activation of the RNase domain²⁰⁶.

ATP-binding pseudokinases with unclear relevance for modulation by ATP-competitive small molecules

For the majority of pseudokinases with demonstrated nucleotide-binding ability, the feasibility of targeting their functions using ATP-competitive compounds remains to be determined. Recently, a type II kinase inhibitor, referred to as compound 1, that binds the nucleotide-binding pocket of the MLKL pseudokinase domain was identified. Upon binding, compound 1 inhibited the translocation of MLKL to the plasma membrane and the induction of necroptosis in response to tumour necrosis factor (TNF), SMAC-mimetic and the pan-caspase inhibitor Q-VD-OPh. These findings led to the hypothesis that the compound interferes with the conformational change induced by RIPK3-mediated phosphorylation of the MLKL activation loop that is necessary for MLKL translocation and oligomerization⁹⁹. Although apo MLKL adopts a DFG-in conformation in crystal structures, it crystallizes in the DFG-out conformation when bound to compound 1, supporting the idea that MLKL signalling could be regulated by conformationally selective ATP-competitive small molecules^{155–157}. Regrettably, compound 1 is not very specific and was found to inhibit several other kinases, including RIPK1¹⁵⁷. Whether a highly selective type II inhibitor of MLKL would inhibit necroptosis, and thus whether ATP-competitive small molecules can inhibit necroptosis through binding to MLKL, remains unclear.

The pseudokinase ILK is overexpressed in multiple types of cancer, including colorectal cancer and NSCLC, in which ILK promotes cell migration and invasion by triggering F-

actin filament bundling^{207–209}. The ILK pseudokinase domain was crystallized in complex with the focal adhesion protein α -parvin both with and without Mg^{2+} -ATP bound in the pseudoactive site²¹⁰. In both structures, ILK and α -parvin interact in a fashion that resembles a canonical kinase–substrate interaction. In this binding mode, the ILK pseudoactive site, including a portion of the activation loop, forms a major part of the interface with α -parvin. The binding interface is nearly identical in the apo and ATP-bound structures, and mutations that disrupt ATP binding have no effect on the interaction with α -parvin or the localization of ILK to focal adhesions²¹¹. Interestingly, another mutation (L207W) in the ILK pseudoactive site that prevents ATP binding markedly impairs the ability of ILK to promote F-actin bundling²⁰⁹. This observation hints at the potential for regulation of ILK function in cell migration using ATP-competitive small molecules.

Another pseudokinase in which disruption of ATP binding does not seem to have a functional consequence is the *T. gondii* pseudokinase ROP5, which contributes to virulence by allosterically inhibiting the immunity-related interferon-inducible GTPase 1 (IIGP1; also known as IRGA6)²¹². There are no noticeable differences in the conformation of ROP5 in the crystal structures of the apo and ATP-bound states of the pseudokinase domain²¹³. The same conformation of the ROP5 pseudokinase domain was also observed in a co-crystal structure of ROP5 in complex with ADP and IIGP1²¹⁴. Thus, the role of nucleotide binding in ROP5-dependent virulence remains poorly understood.

Likewise, the secretory pathway pseudokinase FAM20A, which binds to ATP and, to a lesser extent, to ADP, GTP, CTP and UTP^{112,164}, adopts the same conformation in crystal structures in the apo and ATP-bound states¹⁶⁴. Whether ATP binding plays a role in regulating FAM20A function has yet to be determined. Recent structural studies suggest that ATP-binding stabilizes heterotetramerization of the FAM20A–FAM20C kinase complex (in 2:2 stoichiometry), but the importance of this oligomeric state in signalling remains unknown²¹⁵.

Alternative approaches

For pseudokinases in which nucleotide-binding seems to not affect conformational state or function and for those whose divergent putative nucleotide-binding pockets preclude ligand binding, alternative strategies need to be considered. These approaches could rely on allosteric modulators that use binding sites outside of the canonical nucleotide-binding pocket, molecules that recruit degradation machinery to target pseudokinases and therapeutic antibodies.

Allosteric inhibitors

At present, there are no examples of non-ATP-competitive compounds that modulate pseudokinase function. A growing number of such molecules have been reported for active kinases, demonstrating the feasibility of this strategy for targeting kinase functions. In 2013, the type III inhibitor of MEK1 and MEK2, trametinib, became the first allosteric kinase inhibitor to be approved by the FDA, and since then, several other allosteric kinase inhibitors have entered clinical trials. Type III MEK inhibitors, such as trametinib, bind in a small pocket adjacent to the nucleotide-binding site and stabilize a SRC/CDK-like inactive

conformation of the kinase domain²¹⁶. Thus, trametinib provides an example of conformational selection that is incompatible with MEK1 and MEK2 signalling even when ATP binding is preserved.

The allosteric EGFR inhibitor EAI045 binds selectively to the drug-resistant EGFR-T790M mutant. Like trametinib, EAI045 does not preclude ATP binding because it occupies an adjacent pocket within the active site that only becomes available in the SRC/CDK-like inactive conformation owing to displacement of helix α C away from the active site²¹⁷. Because EAI045 cannot bind to the kinase domain in the active state, its efficacy is greatly improved when used in combination with the anti-EGFR antibody cetuximab. Cetuximab, which inhibits EGFR dimerization, promotes the SRC/CDK-like inactive state of the EGFR kinase and hence improves EAI045 binding²¹⁷, providing an elegant example of a potentially very effective combination therapy.

Another class of allosteric inhibitors explores functionally important sites on kinases that are located distally from the nucleotide-binding pocket. A series of AKT inhibitors, including MK-2206 and AKT inhibitor VIII, bind to the interface between the N-lobe of the kinase domain and the adjacent Pleckstrin homology (PH) domain and thereby lock the AKT kinase domain in an inactive conformation that prevents ATP binding, membrane association and activation loop phosphorylation^{218,219}. Similarly, small molecules that bind to the myristate-binding pocket in the C-lobe of the kinase domain of ABL modulate association of the SRC homology 2 (SH2) and SH3 domains with the kinase domain to control ABL activity. These type IV inhibitors can lead to opposing changes in ABL activity depending on the nature of their effect on inhibition of the kinase domain mediated by the SH2 or SH3 domain. The small molecule GNF-2 locks ABL in an autoinhibited state in which the SH2 and SH3 domains interact tightly with the kinase domain, whereas bulkier ligands, such as the small molecule DPH, activate ABL kinase activity by preventing the SH2 and SH3 domains from adopting the autoinhibited conformation^{220,221}. In addition to its effect on stabilizing an inactive conformation of ABL, GNF-2 also sensitizes ABL to ATP-competitive inhibitors, thereby capitalizing on the allosteric connection between the kinase C-lobe and the active site²²².

For multidomain targets in which the kinase domain is functionally controlled by other domains, effective allosteric inhibitors could exert their effect by binding to sites outside the kinase domain. A precedent for such regulation is set by the small molecule SSR128129E, which binds to the extracellular domain of the fibroblast growth factor receptor (FGFR) and induces a conformational change that biases signalling^{223,224}. Recent studies of RTK and cytokine receptor signalling have further demonstrated the remarkable communication between the conformation of receptor extracellular domains and downstream signalling kinetics and pathway bias^{198,225,226}. While the extracellular domains of RTKs are typically targeted using antibody-based therapeutics, the example of SSR128129E illustrates how small molecules could be used to modulate downstream signalling through binding to the extracellular domain. This approach could therefore be applied to regulate signalling by pseudokinase RTKs.

Allosteric inhibitors have also been successfully used to disrupt intermolecular protein–protein interactions that involve protein kinases. Small molecules that bind to the PIF pocket, located in the N-lobe of the PDK1 kinase domain, disrupt the interaction of PDK1 with substrates that specifically bind this pocket through a conserved hydrophobic motif^{227,228}. A site analogous to the PIF pocket is involved in the receiver interface of the asymmetric dimer formed by HER kinases³³. Small molecules targeting this interface would be expected to prevent asymmetric dimer formation and thereby inhibit HER signalling.

Induced protein degradation

A promising approach for pharmacological targeting of pseudokinases relies on induced protein degradation through proteolysis-targeting chimaeras (PROTACs) or hydrophobic tagging (HyT)²²⁹. PROTACs are bifunctional small molecules that bind to a target protein and recruit a ubiquitin ligase, such as von Hippel–Lindau disease tumour suppressor (VHL) or cereblon, to promote proteasomal degradation of the target. In HyT, a hydrophobic moiety, such as adamantane or tert-butyl carbamate-protected arginine (Boc3Arg), is appended to the target protein to induce degradation through the unfolded protein response. Using ATP-competitive small molecules, PROTACs have been developed for several active protein kinases, such as RIPK2, CDK9 and TBK1, as well as the RTKs EGFR, HER2 and MET^{230,231}. HyT has been used to induce degradation of the pseudokinase HER3²³². In this approach, an adamantane moiety was added to a covalent ATP-competitive HER3 binder, TX1–185-1, to generate TX2–121-1, which induced proteasomal degradation of HER3 and reduced its heterodimerization with HER2 and MET²³² (Fig. 5b). Analogous molecules could be particularly useful for targeting other pseudokinases that are capable of nucleotide binding but whose signalling is not sensitive to conformational changes induced by the binding of ATP analogues.

Small molecules other than PROTAC and HyT bifunctional ligands can also induce protein degradation. For example, ATP-competitive covalent inhibitors of EGFR and HER2, including afatinib and neratinib, destabilize the pseudokinase TRIB2 *in vitro* and induce its ubiquitylation and degradation in cells (Fig. 5c; Table 3). The inhibitors form a covalent adduct with a highly conserved cysteine residue located in helix α C of TRIB2 that is absent in other TRIB pseudokinases²³³. Although the mechanism for the destabilizing effect is not entirely clear, inhibitor binding to TRIB2 was shown to result in displacement of the C-terminal tail from its binding site in the N-lobe of the pseudokinase domain and, in doing so, perhaps interferes with interactions with unknown stabilizing factors²³³. This specific C-terminal-tail-mediated interaction with the N-lobe is a unique feature of TRIB pseudokinases and was first observed in the crystal structure of the closely related pseudokinase TRIB1^{143,233}.

An indirect strategy to induce degradation of a target protein involves the selective inhibition of cellular processes necessary for protein maturation and trafficking. This concept could be especially relevant for pseudokinase RTKs, which, as membrane proteins, must undergo a series of maturation steps as they proceed through the secretory pathway. One of these steps is the insertion of their transmembrane domains into the endoplasmic reticulum membrane, a process mediated by the Sec61 translocon. A class of cyclic peptides known as cotransins

are Sec61 inhibitors that selectively disrupt the insertion of proteins that contain specific signal peptide sequences²³⁴. One of the cotranslins, CT8, prevents Sec61-mediated cotranslational membrane insertion of HER3, leading to HER3 degradation and disruption of HER3 signalling in cancer cells²³⁵. Remarkably, CT8 acts selectively on HER3, but not other HER receptors, owing to unique determinants within the HER3 signal peptide sequence. Future structure–function studies to elucidate the mechanisms that underlie signal peptide recognition by Sec61 and inhibition by cotranslins could lead to the development of molecules that selectively block cotranslational insertion and could be used to target other pseudokinase receptors.

Antibodies against pseudokinase RTKs

Pseudokinase RTKs offer a unique opportunity for targeting using antibody-based approaches. Owing to its role in drug resistance in EGFR-driven and HER2-driven cancers, there has been much interest in developing HER3 antibodies for clinical use²³⁶. Several HER3 antibodies were well tolerated in phase I clinical trials and have been tested in combination with other established therapeutic agents for treatment of patients with advanced or metastatic solid tumours in phase II studies^{237–241} (Table 3). These antibodies, such as seribantumab (MM-121) and lumretuzumab (RG7116), inhibit HER3 by preventing ligand binding and/or its dimerization with other HER receptors^{242,243} (Fig. 5d). Patritumab (U3–1287) is the only HER3 antibody so far to advance to a phase III clinical trial²⁴⁴. Although its mechanism of action remains poorly understood, patritumab promotes HER3 internalization and has been shown to be effective in reducing tumour growth in several tumour cell lines, including cellular models of NSCLC, breast cancer and pancreatic cancer^{245,246}. Despite promising results in a phase II study, however, treatment with a combination of patritumab and the EGFR inhibitor erlotinib failed to increase progression-free survival or overall survival compared with erlotinib alone in patients with advanced NSCLC in a phase III clinical trial^{244,247}.

Another strategy to inhibit HER3 signalling involved efforts to develop bifunctional antibodies that, in addition to HER3, also target its co-receptor EGFR. The dual specificity antibody duligotuzumab (MEHD-7945A) was able to overcome acquired resistance to the EGFR antibody cetuximab or the EGFR kinase inhibitor erlotinib in NSCLC and SCCHN cells. This antibody binds to the inactive ‘tethered’ conformation of both HER3 and EGFR and is believed to block ligand binding^{248,249}. Phase II trials for duligotuzumab, however, failed to improve progression-free survival or the objective response rate when compared with cetuximab treatment in patients with SCCHN or RAS wild-type metastatic colorectal cancer^{250,251}. Subsequently, duligotuzumab was combined with another two-in-one antibody — bH1–44 — that binds to HER2 and vascular endothelial growth factor (VEGF) to create the ‘four-in-one’ antibody FL518, which recognizes EGFR, HER2, HER3 and VEGF. This antibody was more effective than either parental bispecific antibody at inhibiting tumour growth of various cancer cell lines, as well as xenografts²⁵².

Several other antibodies that target pseudokinase RTKs have been reported (Table 3). ROR1, which is expressed in chronic lymphocytic leukaemia (CLL) cells and only rarely in normal adult tissues, is potentially a very selective therapeutic target for the treatment of CLL.

Antibodies against ROR1 have been shown to induce apoptosis of CLL cells but not normal cells²⁵³, and the anti-ROR1 antibody cirmtuzumab (UC-961) is currently being evaluated in several phase I clinical trials²⁵⁴. The pseudokinase ephrin receptor, EPHA10, is also rarely expressed in healthy tissues but is overexpressed in breast and prostate cancers^{255,256}. Treatment with an anti-EPHA10 antibody results in complement-dependent cytotoxicity in prostate cancer cells in an EPHA10-dependent manner²⁵⁶. A bispecific antibody that targets both EPHA10 and the T cell co-receptor CD3 has also been reported to induce T cell-dependent cytotoxicity in cell lines overexpressing EPHA10, as well as in xenograft models²⁵⁷. Finally, the RTK pseudokinase PTK7 was found to be enriched in tumour-initiating cells in patient-derived xenografts (PDXs) of triple-negative breast cancer, ovarian cancer and NSCLC²⁵⁸. Treatment of tumour cells with PF-06647020, an antibody–drug conjugate consisting of an anti-PTK7 antibody and the auristatin microtubule inhibitor Aur0101, resulted in cytotoxicity in vitro and sustained tumour regression in PDX mice²⁵⁸. PF-06647020 is currently being evaluated in phase I trials for patients with advanced solid tumours and metastatic triple-negative breast cancer.

Conclusions

As the functions of pseudokinases continue to be uncovered, the importance of these unusual kinases for normal physiological processes and in disease is becoming increasingly apparent. A landmark example is the EGFR family of receptors, in which signalling by the pseudokinase HER3 underlies resistance to inhibitors that target the active receptors EGFR and HER2, suggesting that only combination therapy directed towards multiple EGFR family members will ultimately be effective. Although several HER3-targeted antibodies have already been tested in clinical trials in combination with small molecules or antibodies that target EGFR or HER2 for the treatment of advanced solid tumours, so far, these combinations have not demonstrated greater efficacy than existing treatment regimens. More careful selection of study participants or alternative treatment modalities aimed at HER3 might be necessary to achieve clinical benefits.

In this Review, we discuss several examples in which modulation of pseudokinase signalling can be achieved through a pocket that, in active kinases and some pseudokinases, binds nucleotides. The remarkable structural diversity of these pockets in pseudokinases provides an opportunity to achieve a high degree of specificity in pseudokinase-directed small molecules. For pseudokinases that do not bind nucleotides, the structural features of some of the ‘pseudo’-nucleotide-binding pockets might still offer this possibility. The underlying goal of these pseudokinase-binding molecules is to disrupt a functional conformation of the pseudokinase domain that may mediate protein–protein interactions that are necessary for signal propagation. Alternatively, a small molecule can be used to mediate target degradation. This strategy is particularly appealing for targeting pseudokinases. The degradation-inducing molecules discussed in this article target nucleotide-binding pockets in pseudokinases and active kinases, but small molecules that bind to distal allosteric sites in the pseudokinase domain or adjacent domains could also be used to develop molecules that induce degradation and inhibit pseudokinase signalling. Finally, interfering with the function of cell surface-exposed pseudokinases has been successful in preclinical and early clinical

studies and could be further used to develop antibody-based, pseudokinase-directed therapeutics.

Recent reports of the unexpected effects of small-molecule inhibitors on the noncatalytic functions of active kinases emphasize the need to carefully consider the physiological consequences of kinase inhibition in cells. Although the emerging paradoxical effects of active kinase inhibitors are a cautionary tale, they should also guide the design of molecules that can modulate pseudokinase signalling. Structural and mechanistic studies of pseudokinases show striking parallels between pseudokinase regulation and that of their active counterparts. This finding indicates that much of what we have learned from drugging active kinases can be applied to targeting pseudokinases and vice versa. Encouragingly, the chemical space for pseudokinase drug design is likely much larger than that of active kinases owing to the uniqueness of the architectures of pseudoactive sites, a consideration to keep in mind when screening for novel small molecules that bind pseudokinases.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgements

The authors sincerely apologize to all colleagues whose work was omitted in this Review owing to space constraints. N.J. and J.E.K. are supported by a grant from the National Institute of General Medical Sciences to N.J. (R01 GM109176), Susan G. Komen Foundation Training Grant to N.J. (CCR14299947), National Science Foundation Graduate Research Fellowship to J.E.K. and University of California at San Francisco (UCSF) Discovery Fellowship to J.E.K.

Glossary

<i>Salt bridge</i>	A non-covalent interaction between oppositely charged residues.
<i>Type II inhibitor</i>	An ATP-competitive small molecule that binds to the inactive DFG (Asp-Phe-Gly)-out conformation of a kinase.
<i>Type I inhibitor</i>	An ATP-competitive small molecule that binds to the active DFG (Asp-Phe-Gly)-in conformation of a kinase.
<i>Protomers</i>	Individual subunits of a multimeric protein complex. In the context of a BRAF homodimer, a protomer would be a single BRAF monomer.
<i>Apo</i>	A state of a protein when it has no ligands bound.
<i>Type III inhibitor</i>	A small-molecule kinase inhibitor that binds to an allosteric site adjacent to the ATP-binding pocket and does not interfere with ATP binding.
<i>Type IV inhibitors</i>	Small-molecule kinase inhibitors that bind to allosteric sites distal from the ATP-binding site.

Sec61 translocon

A protein complex that mediates insertion of the transmembrane *domains of membrane proteins into the endoplasmic reticulum membrane.*

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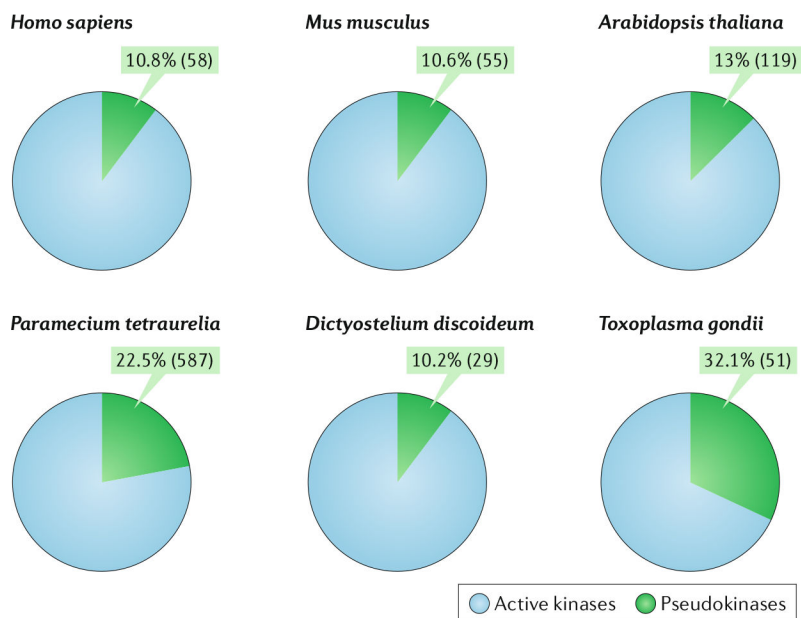


Fig. 1: Prevalence of pseudokinases in the kinomes of diverse species.

Pie charts showing the percentages of active kinases (blue) and pseudokinases (green) in the kinomes of the indicated organisms. Pseudokinases are defined as kinases that carry mutations in one or more conserved catalytic motifs (the β 3 lysine within the VAIK (Val-Ala-Ile-Lys) motif, the aspartate in the DFG (Asp-Phe-Gly) motif and the aspartate in the HRD (His-Arg-Asp) motif).

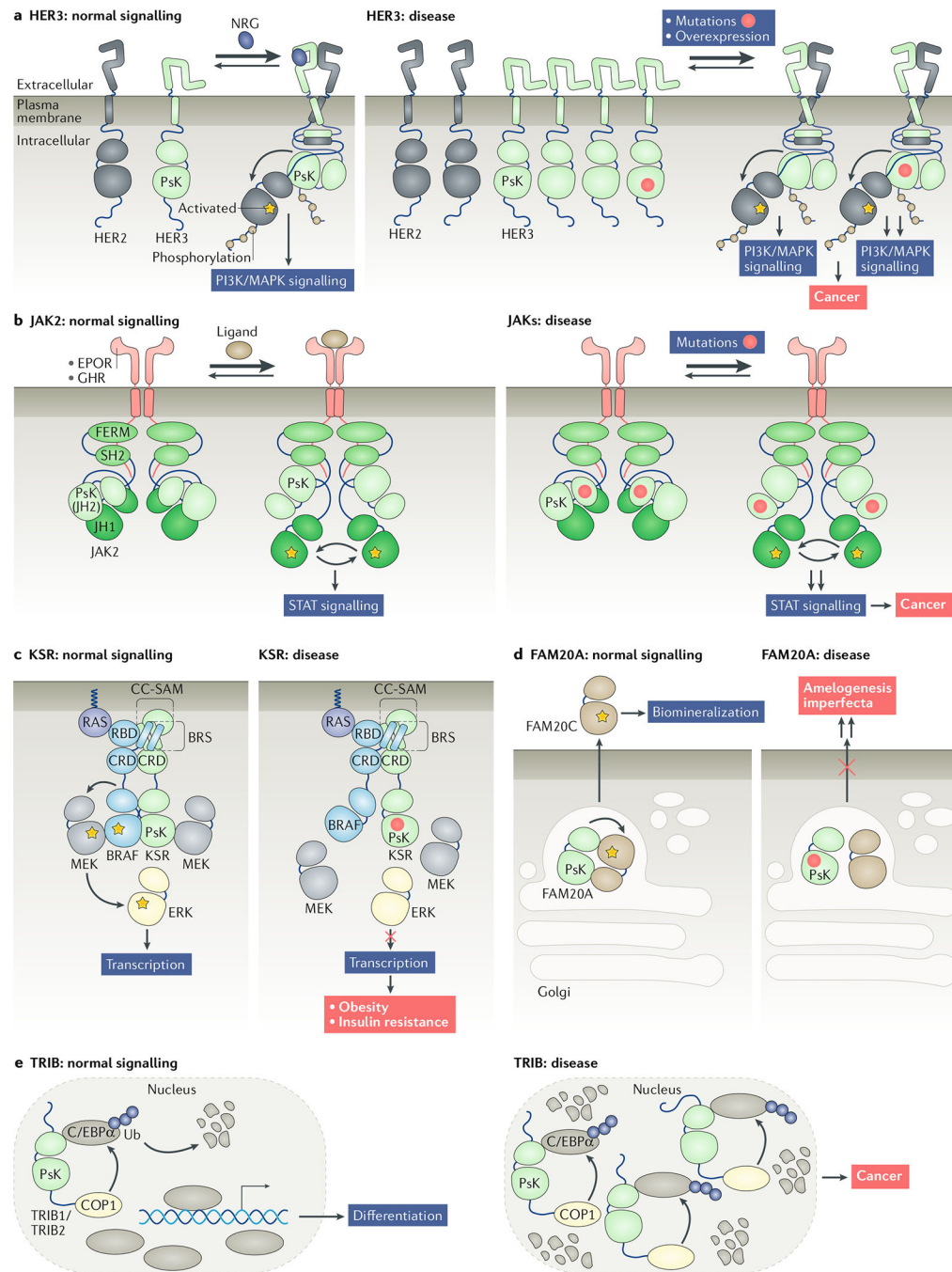


Fig. 2: Dysregulation of pseudokinase signalling in disease.

a | In normal cells, human epidermal growth factor receptor 3 (HER3) allosterically activates other HER kinases through heterodimerization in a ligand-dependent manner (left panel). Overexpression or mutation of HER3 drives ligand-independent association with its dimerization partners, such as HER2, and activation of signalling pathways that promote tumorigenesis (right panel). **b** | In normal cells, Janus kinase 2 (JAK2) associates with its cognate receptor at the plasma membrane, and the JAK homology 2 (JH2) pseudokinase domain of JAK2 allosterically inhibits the JH1 kinase domain. This inhibition is relieved

upon ligand binding to the receptor, allowing transphosphorylation and activation of the kinase domain (left panel). Mutations in the pseudokinase domain associated with cancers, such as myeloproliferative neoplasms, are believed to disrupt the pseudokinase–kinase domain interaction, causing constitutive activation of the kinase domain (right panel). **c** | Under normal conditions, kinase suppressor of RAS (KSR) allosterically activates BRAF to promote phosphorylation of MEK and activate mitogen-activated protein kinase (MAPK) signalling. KSR also interacts directly with MEK, and this interaction enhances the allosteric activator function of KSR (left panel). Mutations associated with obesity and insulin resistance impair the ability of KSR to activate the MAPK pathway by disrupting its interactions with MEK and BRAF (right panel). **d** | Under normal conditions, FAM20A and FAM20C localize to the Golgi apparatus. FAM20A allosterically activates FAM20C, which becomes secreted and phosphorylates extracellular proteins that are important for biomineralization and proper enamel development (left panel). Mutations in FAM20A linked to amelogenesis imperfecta fail to activate FAM20C or promote FAM20C secretion (right panel). **e** | The transcription factor CCAAT enhancer-binding protein- α (C/EBP α) promotes myeloid cell differentiation. Tribbles homologue 1 (TRIB1) and TRIB2 interact with C/EBP α and the E3 ubiquitin ligase COP1 to mediate ubiquitylation and proteasomal degradation of C/EBP α (left panel). Overexpression of TRIB1 or TRIB2 induces leukaemogenesis by causing excessive degradation of C/EBP α in a COP1-dependent manner (right panel). CRD, cysteine-rich domain; NRG, neuregulin; PI3K, phosphoinositide 3-kinase; STAT, signal transducer and activator of transcription.

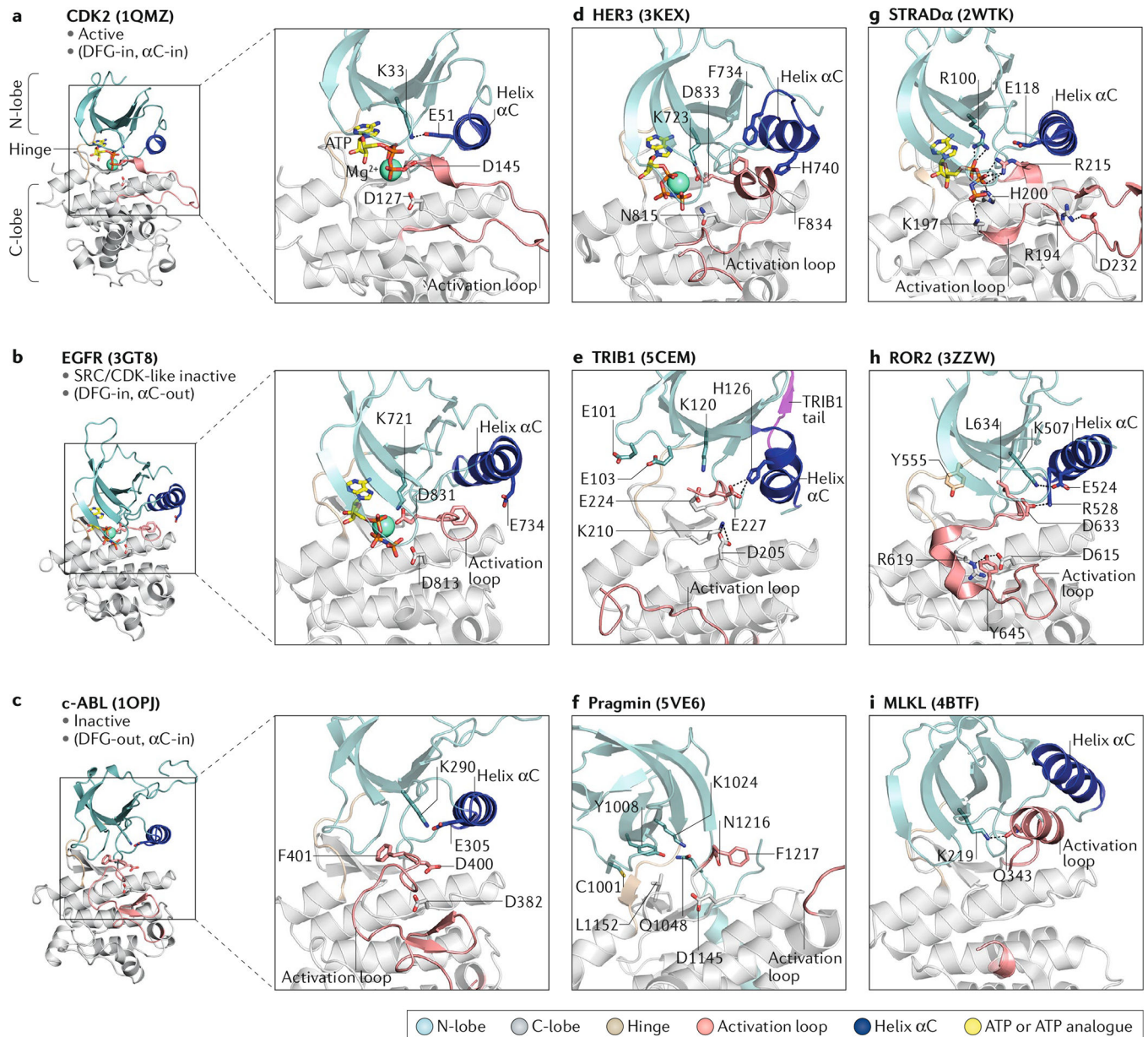


Fig. 3: Structural features of pseudokinases.

Left panels show crystal structures of the CDK2, epidermal growth factor receptor (EGFR) and ABL kinase domains in the active, SRC/CDK-like inactive and inactive conformations, respectively. Insets show zoomed views of the active sites. Right panels show zoomed views of the pseudoactive sites in the crystal structures of the indicated pseudokinases. **a** | In the active conformation (CDK2 is shown), the β 3 lysine (K33) forms a salt bridge with E51 in helix α C, and the DFG (Asp-Phe-Gly) aspartate (D145) coordinates Mg^{2+} in the active site. **b** | In the SRC/CDK-like inactive conformation (EGFR is shown), helix α C is shifted away from the active site, preventing a salt bridge between the β 3 lysine (K721) and E734. **c** | In the inactive conformation (ABL is shown), the DFG motif is flipped so that phenylalanine (F401) blocks nucleotide binding. **d** | Helix α C in human epidermal growth factor receptor 3

(HER3) is partially unwound and adopts a SRC/CDK-like inactive conformation. **e** | Helix α C is unusually short in TRIB1 and adopts a kink, resulting in a shallow nucleotide-binding pocket. **f** | Pragma has a disordered helix α C and occluded nucleotide-binding pocket. **g** | STE20-related adaptor- α (STRAD α) coordinates ATP using positively charged residues. **h** | The ROR2 DLG motif is shifted ~ 4 Å compared with canonical DFG motifs and interacts with helix α C, pushing it away from the pseudoactive site. **i** | Mixed lineage kinase domain-like protein (MLKL) has an unusual activation loop helix that interacts with the $\beta 3$ lysine (K219). The corresponding Protein Data Bank codes for each structure shown are indicated in parentheses. C-lobe, carboxyl-terminal lobe; N-lobe, amino-terminal lobe.

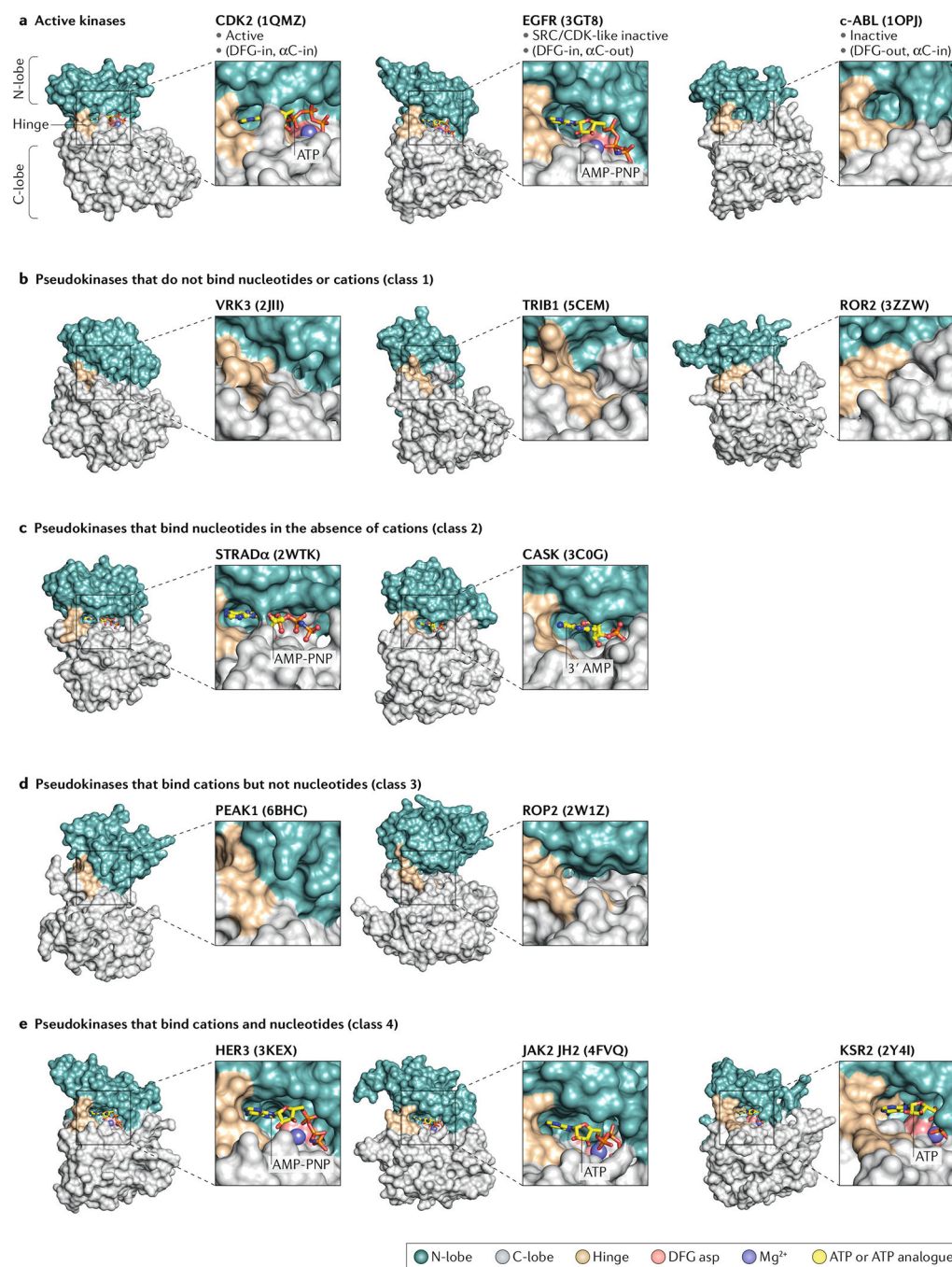


Fig. 4: Accessibility of the putative nucleotide-binding pocket in different classes of pseudokinases.

Surface representations of crystal structures of active kinases in the active DFG-in (CDK2), SRC/CDK-like inactive (EGFR) and inactive DFG-out conformations (c-ABL) (part **a**), pseudokinases that do not bind nucleotides or cations (part **b**), pseudokinases that bind nucleotides in the absence of cations (part **c**), pseudokinases that bind cations but not nucleotides (part **d**) and pseudokinases that bind nucleotides and cations (part **e**). For each structure, bound ATP or ATP analogues are shown as sticks, while Mg²⁺ ions are shown as

spheres. Insets show zoomed views of the putative nucleotide-binding pocket for each kinase or pseudokinase. The corresponding Protein Data Bank codes for each crystal structure shown are indicated in parentheses. α C, helix α C; AMP-PNP, nonhydrolysable ATP analogue; C-lobe, carboxyl-terminal lobe; EGFR, epidermal growth factor receptor; HER3, human EGFR; N-lobe, amino-terminal lobe; JAK2, Janus kinase 2; JH2, JAK homology 2; KSR2, kinase suppressor of RAS 2; ROP2, rhoGTPase activating protein 2; STRAD α , STE20-related adaptor- α .

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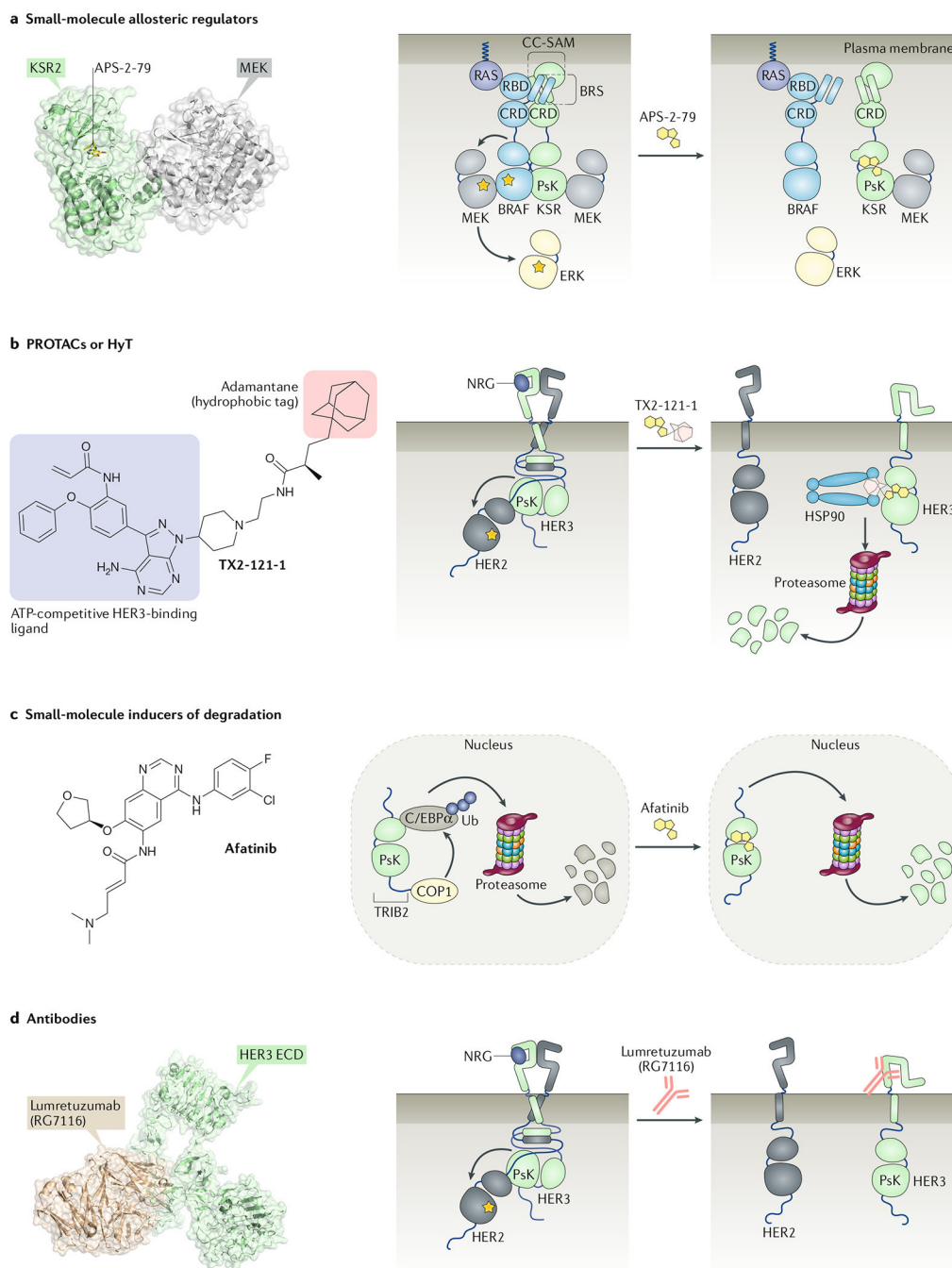


Fig. 5: Strategies for pharmacological targeting of pseudokinases.

a | Small molecules can be used to allosterically modulate the conformation and interactions of pseudokinases, as illustrated by APS-2-79, an ATP-competitive inhibitor of kinase suppressor of RAS 2 (KSR2), that stabilizes an inactive conformation of KSR2 that is incompatible with heterodimerization with RAF and that blocks RAF-dependent phosphorylation sites on MEK. A crystal structure of KSR2 bound to APS-2-79 and MEK is shown in the left panel. **b** | Proteolysis-targeting chimaeras (PROTACs) or hydrophobic tagging (HyT) can be used to induce degradation of a protein of interest. The human

epidermal growth factor receptor 3 (HER3) inhibitor TX2–121-1 consists of a portion that binds covalently to the HER3 nucleotide-binding pocket (blue) connected to adamantane (red), a hydrophobic moiety that mimics the presence of an unfolded protein. Binding of TX2–121-1 leads to proteasomal degradation of HER3, a process that is aided by chaperones, including HSP70 and HSP90. **c** | Small molecules other than the bifunctional ligands employed for PROTAC-mediated or HyT-mediated protein degradation can be used to destabilize pseudokinases and induce their degradation. Covalent inhibitors of epidermal growth factor receptor (EGFR) and HER2, such as afatinib, destabilize the pseudokinase TRIB2 and promote its proteasomal degradation. **d** | Antibodies can be used to target the extracellular domains (ECDs) of receptor tyrosine kinases that possess pseudokinase domains. The monoclonal antibody lumretuzumab (RG7116) locks the HER3 ECD in an inactive conformation that prevents ligand binding. A crystal structure of an antigen-binding fragment, derived from lumretuzumab, bound to the HER3 ECD is shown in the left panel. C/EBP α , CCAAT enhancer-binding protein- α ; CRD, cysteine-rich domain; NRG, neuregulin.

Table 1.

Known roles of pseudokinases in disease

Pseudokinase	Links to disease	Relevant pseudokinase domain mutations
ADCK3	Mutations associated with cerebellar ataxia result in defects in coenzyme Q ₁₀ synthesis	Y514C
ANP-A (encoded by <i>NPR1</i>)	Polymorphisms associated with increased left ventricular mass index in essential hypertension; deletion in 5' flanking region associated with increased susceptibility to essential hypertension or left ventricular hypertrophy	NA
ANP-B (encoded by <i>NPR2</i>)	Loss-of-function mutations associated with acromesomelic dysplasia, Maroteaux type	Y708C, R776W
BUBR1	Mutations associated with mosaic variegated aneuploidy; overexpression associated with tumour cell proliferation in gastric cancer and chromosomal instability in bladder cancer	R727C, R814H, I909T, L1012P
CASK	Mutations associated with X-linked mental retardation and FG syndrome	R28L, Y268H
DIA1R (encoded by <i>CXORF36</i>)	Mutations associated with X-linked mental retardation; deletions within <i>CXORF36</i> associated with autism spectrum disorders	R128K
EPHA10	Overexpression associated with prostate cancer and correlated with lymph node metastasis and stage progression in breast cancer	NA
EPHB6	Downregulated in metastatic melanoma; expression level associated with tumour stage and survival in neuroblastoma and ovarian serous carcinoma; deletion mutation enhances cell migration and promotes a pro-metastatic phenotype in NSCLC cells	NA
FAM20A	Mutations associated with amelogenesis imperfecta prevent allosteric activation of FAM20C and exhibit reduced secretion	L173R, G331D, D403N
GCN2	Promotes survival and proliferation in fibrosarcoma cells; genetic deletion improved synaptic plasticity and spatial memory in a mouse model of Alzheimer disease; promotes efficacy of yellow fever vaccine; mutations associated with pulmonary veno-occlusive disease	NA
HER3 (also known as ERBB3)	Increased expression and persistent phosphorylation of HER3 contribute to drug resistance in cancer types such as breast and lung cancer; mutations promote oncogenesis and are found in several tumour types including breast, lung and gastrointestinal cancer; downregulated in dorsolateral prefrontal cortex in schizophrenia; overexpressed in Charcot-Marie-Tooth type 1 disease	V695M, Q790R, S827I, E909G
HSER (encoded by <i>GUCY2C</i>)	Loss-of-function mutations linked to meconium ileum; activating mutation linked to familial diarrhoea syndrome; mRNA expression in CRC linked to time to recurrence and disease-free survival; suppresses intestinal tumour formation by inhibiting AKT	NA
ILK	Overexpression promotes cell migration and invasion in lung cancer and CRC	NA
IRAK2	Hypofunctional mutations associated with reduced survival in CRC and reduced hepatitis C clearance	R214G, L392V
IRAK3	Overexpression associated with deactivation of inflammatory response in monocytes upon exposure to tumour cells; mutations associated with early-onset, persistent asthma	L400V, R429Q
JAK1 JH2	Mutations associated with acute leukaemias	A634D, H647Y, L653F, V658F, R724H, T782M, L783F
JAK2 JH2	Mutations associated with myeloproliferative neoplasms, such as polycythaemia vera, and acute leukaemias	K607N, L611S, V617F, V717F
JAK3 JH2	Inhibitory mutations associated with severe combined immunodeficiency; activating mutations associated with acute leukaemias	A572V, A573V, M576L, A593T, V715I, V722I
KSR1	KSR1-null mice exhibit impaired tumorigenesis in RAS-driven cancers	NA
KSR2	Mutations associated with obesity and insulin resistance; overexpression promotes anchorage-independent growth and transformation of oncogenic RAS-mutant cells	P662L, E667V, R684C, I801L, G816D, R818Q, R823H, D843N, S904L
MLKL	Required for necroptosis; overexpressed in autoimmune hepatitis	NA

Pseudokinase	Links to disease	Relevant pseudokinase domain mutations
NRBP1	Downregulated in multiple cancers, including lung and colon adenocarcinomas; low expression levels in lung tumours correlate with reduced survival	NA
NRBP2	Downregulated in hepatocellular carcinoma; high expression levels correlated with greater chemosensitivity and better prognosis	NA
PEAK1 (also known as SGK269)	Overexpressed in pancreatic cancer and a subset of breast cancers; required for KRAS-induced cell expansion in pancreatic cancer	NA
POMK (also known as SGK196)	Required for Lassa virus entry; loss-of-function mutations associated with Walker–Warburg syndrome, neuromuscular disorders and congenital muscular dystrophy	L137R, Q258R
Pragmin (also known as SGK223 or PRAG1)	Overexpressed in pancreatic cancer; required for SRC-mediated invasion in CRC	NA
PTK7	Mutations lead to idiopathic scoliosis and neural tube defects; overexpression associated with lung and breast cancer; downregulated in metastatic melanoma and ovarian cancer	NA
PXK	Genetic variants that reduce B cell receptor internalization associated with systemic lupus erythematosus	NA
RETGC1 (encoded by <i>GUCY2D</i>)	Mutations associated with Leber congenital amaurosis and autosomal dominant cone–rod retinal dystrophy	F589S
RETGC2 (encoded by <i>GUCY2F</i>)	Mutations associated with pancreatic, lung and breast cancers	K672T
RNase L	Mediates innate immunity against viral and bacterial infections; overexpression observed in chronic fatigue syndrome; mutations associated with elevated risk of prostate cancer	R462Q
ROR1	Overexpressed in several types of cancer, including CLL, ALL, gastric carcinoma and NSCLC	NA
ROR2	Germline deletions associated with autosomal dominant brachydactyly type B; loss-of-function mutations associated with autosomal recessive Robinow syndrome; overexpression associated with osteosarcoma and renal cell carcinoma	N620K
RPS6KL1	Knockdown reduces cell survival of cervical carcinoma cells	NA
RYK	Inhibition of axon regeneration following spinal cord injury; overexpressed in epithelial ovarian cancer and associated with decreased overall survival	NA
S6K 1 (encoded by <i>RPS6KC1</i>)	Fusion of PX domain with AKT3 results in constitutively activated oncogenic fusion protein that promotes tumour growth of HR ⁺ breast cancer cells	NA
SCYL1	Frameshift mutations associated with recessive form of spinocerebellar neurodegeneration and a hepatocerebellar neuropathy syndrome	NA
SCYL2	Reduces release of HIV-1 by promoting Vpu dephosphorylation	NA
SCYL3	Oncogenic fusion of TRKA kinase domain with SCYL3 pseudokinase domain and HEAT repeats is associated with CRC and promotes cell proliferation	NA
STK31 (also known as SGK396)	Overexpressed in CRC; inhibits differentiation; promotes cell migration and invasion	NA
STK40 (also known as SGK495)	Knockdown reduces cell viability in cervical carcinoma cells	NA
STRAD α	Allosterically activates tumour suppressor LKB1; deletion of exons 9–13 associated with PMSE	NA
STRAD β	Allosterically activates tumour suppressor LKB1	NA
STYK1	Overexpressed in castration-resistant prostate cancer; low expression correlates with improved survival in hepatocellular carcinoma and decreased cell growth, migration and invasion	NA
TBCK	Mutations associated with infantile syndromic encephalopathy, intellectual disability and hypotonia	NA
Titin	Mutations associated with cardiomyopathy	NA
TRIB1	Overexpression associated with AML and prostate cancer; gain-of-function mutation associated with acute megakaryocytic leukaemia; polymorphisms associated with altered lipid metabolism and cardiovascular disease	R107L

Pseudokinase	Links to disease	Relevant pseudokinase domain mutations
TRIB2	Overexpressed in AML, lung cancer and liver cancer; promotes drug resistance in several cancer types, including melanoma and breast and renal cancers	NA
TRIB3	Gain-of-function mutation associated with insulin resistance; overexpression associated with lung, colon and breast cancers; overexpression associated with Parkinson disease	Q84R
TRRAP	Necessary for MYC and E1A-mediated transformation	NA
TYK2 JH2	Inhibitory mutations associated with autoimmune diseases, including systemic lupus erythematosus, Crohn's disease and psoriasis; required for amyloid- β -induced neuronal apoptosis	I684S
ULK4	Deletions of exons 21–34 and 33–34 in the <i>ULK4</i> gene associated with schizophrenia	NA
VRK3	Phosphorylation of VRK3 by CDK5 protects neuronal cells from oxidative stress-induced cell death by inhibiting ERK	NA

A complete table including references can be found in the supplementary information. ALL, acute lymphocytic leukaemia; AML, acute myeloid leukaemia; ANP-A, atrial natriuretic peptide receptor type A; CDK5, cyclin-dependent kinase 5; CLL, chronic lymphocytic leukaemia; CML, chronic myeloid leukaemia; CRC, colorectal cancer; DIA1R, deleted in autism-related protein 1; EPHA10, pseudokinase ephrin receptor; HER, human epidermal growth factor receptor; HR, hormone receptor; HSER, heat-stable enterotoxin receptor; ILK, integrin-linked protein kinase; IRAK2, interleukin-1 receptor-associated kinase-like 2; JAK, Janus kinase; JH2, JAK homology 2; KSR, kinase suppressor of RAS; MLKL, mixed lineage kinase domain-like protein; NA, not applicable; NRBP1, nuclear receptor-binding protein 1; NSCLC, non-small-cell lung cancer; PMSE, polyhydramnios, megalencephaly, symptomatic epilepsy; POMK, protein *O*-mannose kinase; PTK7, protein tyrosine kinase 7; RETGC1, retinal guanylyl cyclase 1; RPS6KL1, ribosomal protein S6 kinase-like 1; RYK, receptor-like tyrosine kinase; SCYL1, SCY-like protein 1; STK, serine/threonine-protein kinase; STRAD α , STE20-related adaptor- α ; TRRAP, transformation/transcription domain-associated protein; TRIB1, Tribbles homologue 1.

Table 2.

Classes of pseudokinases

Pseudokinase	Degraded glycine-rich loop	VAIK motif	HRD motif	DFG motif	Crystal structures	Refs
<i>Class 1 — pseudokinases that do not bind nucleotides or cations</i>						
BIR2 (<i>A. thaliana</i>)	Y	Y	N	Y	4L68	259
BUBR1	Y	Y	Y	Y	NA	159
GCN2	Y	N	N	Y	NA	159
IRAK3	N	Y	N	Y	NA	159
MviN (<i>M. tuberculosis</i>)	Y	N	N	N	3OTV	159-160
NRBP1	Y	N	N	N	NA	159
Pragmin	Y	Y	Y	N	5VE6, 6EWX	145-146-159
PTK7	N	Y	Y	N	NA	159
ROP8 (<i>T. gondii</i>)	Y	N	N	N	3BYV	166
ROR1	N	Y	Y	Y	NA	159
ROR2	N	Y	Y	Y	3ZZW, 4GT4	154
RYK	N	Y	Y	Y	NA	159
SCYL1	Y	N	N	N	NA	159
STK40	Y	Y	Y	N	5L2Q	159-260
Titin	N	Y	Y	N	4JNW, 1TKI	261-262
TRIB1	Y	Y	Y	N	5CEK, 5CEM, 6DC0	120-143
VRK3	Y	Y	N	N	2JII	147
<i>Class 2 — pseudokinases that bind nucleotides in the absence of cations</i>						
CASK	N	Y	Y	N	3C0G, 3C0H, 3C0I, 3MFR (4 M), 3MFS (4 M), 3MFU (4 M), 3TAC	163-263-264
EPHB6	N	N	N	N	NA	159
FAM20A	Y	Y	Y	Y	5WRR, 5WRS, 5YH2, 5YH3	112-164-215
MLKL	Y	Y	N	N	4BTF, 4M67, 4M68, 4M69, 4MWI, 5KNJ, 5K01	97-155-156-157
STRAD α	N	N	N	N	2WTK, 3GNI	152-153
TRIB2	Y	Y	Y	N	NA	162
TRIB3	Y	Y	Y	N	NA	162
ULK4	N	N	Y	N	NA	159
<i>Class 3 — pseudokinases that bind cations but not nucleotides</i>						
PEAK1	Y	Y	Y	N	6BHC	144-159
ROP2 (<i>T. gondii</i>)	Y	N	Y	N	2W1Z, 3DZO	159-165-166
<i>Class 4 — pseudokinases that bind to nucleotides and cations</i>						
ADCK3	Y	Y	Y	Y	4PED	265
ANP-A	N	Y	N	Y	NA	266

Pseudokinase	Degraded glycine-rich loop	VAIK motif	HRD motif	DFG motif	Crystal structures	Refs
BSK8 (<i>A. thaliana</i>)	Y	Y	N	N	4I92, 4I93, 4I94	267
HER3	N	Y	N	Y	3KEX, 3LMG, 4OTW, 4RIW, 4RIX (Q790R), 4RIY (E909G)	30· 32· 151
HSER	Y	Y	N	Y	NA	268
ILK	Y	Y	N	Y	3KMU, 3KMW, 3REP, 6MIB (L207W)	209· 211
IRAK2	Y	Y	N	N	NA	269
JAK1 JH2	N	Y	N	Y	4L00, 4L01 (V685F)	270
JAK2 JH2	N	Y	N	Y	4FVP, 4FVQ, 4FVR (V617F), 5I4N (E596A or V617F), 5USZ, 5UTO, 5UT1, 5UT2, 5UT3, 5UT4, 5UT5, 5UT6	169· 171· 203· 204· 271
KSR1	N	N	Y	Y	NA	103
KSR2	N	N	Y	Y	2Y4I, 5KKR	172· 199
PAN3	Y	Y	N	N	4BWK, 4BWX, 4BWP, 4CYI, 4CYJ, 4CZY, 4XR7	272· 273· 274· 275
POMK	Y	N	Y	Y	5GZA, 5GZ8, 5GZ9	276· 277· 278
RNase L	Y	Y	Y	Y	4OAV, 4OAU, 4O1P, 4O1O	149· 150· 205
ROP5B (<i>T. gondii</i>)	N	Y	N	Y	3Q5Z, 3Q60, 4LV5	213· 214
STKLD1	N	Y	N	Y	NA	159
TYK2 JH2	N	Y	N	Y	3ZON, 4OLI, 4WOV, 5TKD	93· 200· 201

All protein structures are as per the Protein Data Bank. ANP-A, atrial natriuretic peptide receptor type A; *A. thaliana*, *Arabidopsis thaliana*; DFG, Asp-Phe-Gly; HER, human epidermal growth factor receptor; HRD, His-Arg-Asp; HSER, heat-stable enterotoxin receptor; ILK, integrin-linked protein kinase; IRAK2, interleukin-1 receptor-associated kinase-like 3; JAK, Janus kinase; JH2, JAK homology 2; KSR, kinase suppressor of RAS; *M. tuberculosis*, *Mycobacterium tuberculosis*; MLKL, mixed lineage kinase domain-like protein; NA, not available; NRBP1, nuclear receptor-binding protein 1; POMK, protein *O*-mannose kinase; PTK7, protein tyrosine kinase 7; ROP8, rhopty protein 8; RYK, receptor-like tyrosine kinase; SCYL1, SCY-like protein 1; STK, serine/threonine-protein kinase; STRAD α , STE20-related adaptor- α ; *T. gondii*, *Toxoplasma gondii*; TRIB1, Tribbles homologue 1; VAIK, Val-Ala-Ile-Lys.

Table 3.

Known modulators of pseudokinase function

Molecule or antibody name	Type	Mechanism of action	Refs
<i>EPHA10</i>			
BsAb	mAb	Bispecific antibody that targets EPHA10 and CD3; induces redirected T cell-mediated lysis of EPHA10-overexpressing cells	257
Db-1 and Db-2	Diabody	Heterodimeric (Db-1) and homodimeric (Db-2) diabodies that target EPHA10 and CD3; induce redirected T cell-mediated lysis of EPHA10-overexpressing cells	279
<i>HER3</i>			
Elgemtumab (LJM716)	mAb	Binds to inactive conformation of ECD but does not prevent ligand binding; inhibits ligand-dependent and ligand-independent HER3 signalling	280
Duligotuzumab (MEHD-7945A)	mAb	Dual specificity antibody that recognizes the inactive conformations of the HER3 and EGFR ECDs; blocks ligand binding	248-249
Seribantumab (MM-121)	mAb	Inhibits ligand-induced phosphorylation of HER3	242
Patritumab (U3-1287 or AMG-888)	mAb	Promotes receptor internalization; inhibits ligand-induced HER3 signalling	245-246-281
U3-1402	ADC	ADC consisting of patritumab conjugated to the topoisomerase I inhibitor DX-8951	282
Lumretuzumab (RG7116)	mAb	Binds to inactive conformation of ECD and blocks ligand binding; inhibits ligand-induced HER3 signalling; exhibits enhanced ADCC due to glycoengineering of Fc moiety	243
FL518	mAb	Four-in-one antibody made by combining duligotuzumab and bH1-44 that recognizes EGFR, HER2, HER3 and VEGF; inhibits ligand-dependent and ligand-independent signalling	252
AV-203	mAb	Blocks ligand binding and prevents dimerization with HER2; inhibits ligand-dependent and ligand-independent HER3 signalling	283
GSK2849330	mAb	Disrupts ligand-dependent HER3 signalling; engineered to exhibit enhanced ADCC and cell division cycle	284
KTN3379	mAb	Prevents HER2-HER3 heterodimerization; inhibits both ligand-dependent and ligand-independent HER3 signalling	285
REGN1400	mAb	Blocks ligand binding	286
MCLA-128	mAb	Bispecific antibody targeting both HER2 and HER3 that exhibits enhanced ADCC	287
TX2-121-1	Small molecule	Irreversible ATP-competitive inhibitor that forms covalent adduct with Cys721 in HER3; induces degradation of HER3 and interferes with heterodimerization with HER2 and MET	232
Bosutinib	Small molecule	ATP-competitive SRC and ABL inhibitor that promotes HER3 heterodimerization with HER2 and promotes proliferation of HER2 ⁺ breast cancer cells	194
CT8	Small molecule	Binds Sec61 translocon and interferes with cotranslational insertion of HER3 into the ER, resulting in HER3 degradation	235
<i>JAK2 JH2</i>			
JNJ-7706621	Small molecule	ATP-competitive pan-CDK and Aurora A and Aurora B inhibitor that binds to both JAK2 JH1 and JH2	203-204
AT-9283	Small molecule	ATP-competitive Aurora A, Aurora B, JAK2 and JAK3 inhibitor that binds to both JAK2 JH1 and JH2	203
<i>KSR2</i>			
APS-2-79	Small molecule	ATP-competitive inhibitor that stabilizes an inactive conformation of the pseudokinase domain; prevents heterodimerization with RAF kinases and stabilizes inactive complex with MEK	199

Molecule or antibody name	Type	Mechanism of action	Refs
ASC-24 <i>MLKL</i>	Small molecule	ATP-competitive inhibitor that blocks KSR kinase activity in vitro but does not prevent heterodimerization with RAF kinases	172
Compound 1	Small molecule	ATP-competitive VEGFR and RIPK1 inhibitor that stabilizes DFG-out conformation of MLKL pseudokinase domain; inhibits necroptosis	99-157
Compound 4 <i>PTK7</i>	Small molecule	ATP-competitive small molecule that stabilizes DFG-in conformation of MLKL pseudokinase domain; more selective for MLKL than compound 1 but fails to inhibit necroptosis	157
PF-06647020 <i>RNase L</i>	ADC	ADC consisting of the anti-PTK7 mAb h6M24 conjugated to the auristatin microtubule inhibitor Aur0101	258
Sunitinib	Small molecule	ATP-competitive inhibitor of PKR, PDGFR and VEGFR; inhibits RNase L activity and dimerization	206
Flavonols <i>ROR1</i>	Small molecule	ATP-competitive activators of yeast IRE1 RNase activity; inhibit RNase L activation	206
3B8, 1C11, 1D8, 4C10, 4A7	mAb	mAbs raised against immunoglobulin (3B8), CRD (1C11, 1D8) and KNG (4A7, 4C10) domains of ROR1 ECD; induce apoptosis of patient-derived CLL cells	253
Cirmtuzumab (UC-961)	mAb	Inhibits WNT5a-induced activation of RAC1	288
ROR1-CAR <i>RYK</i>	CAR	ROR1-specific CAR generated based on a mAb that binds to the ECD of ROR1; T cells transduced with ROR1-CAR selectively lyse ROR1-overexpressing cancer cells	289
RWD1 <i>TRIB2</i>	mAb	Binds to WIF domain of ECD; inhibits WNT5A-induced signalling and neurite outgrowth	290
Afatinib	Small molecule	Irreversible ATP-competitive inhibitor of EGFR and HER2; forms covalent adduct with Cys96 in TRIB2; destabilizes TRIB2 in vitro and induces its degradation in cells	233
Neratinib, osimertinib	Small molecule	Irreversible ATP-competitive inhibitors of EGFR and HER2; destabilize TRIB2 in vitro	233
Lapatinib, TAK-285, GW693881A	Small molecule	Reversible ATP-competitive inhibitors of EGFR and HER2; stabilize TRIB2 in vitro	233
GW804482X <i>TYK2 JH2</i>	Small molecule	Reversible ATP-competitive inhibitor of PLK; destabilizes TRIB2 in vitro	233
Compound 1, BMS-066	Small molecule	ATP-competitive inhibitor that blocks TYK2-mediated signalling through IL-23R in T cells	200
Compound 35	Small molecule	ATP-competitive inhibitor that blocks TYK2-mediated signalling through IL-23R in T cells with greater selectivity and metabolic stability than compound 1	201
BMS-986165	Small molecule	ATP-competitive inhibitor that blocks TYK2-mediated signalling through IL-12, IL-23 and type I interferon signalling	202

ADC, antibody–drug conjugate; ADCC, antibody-dependent cell-mediated cytotoxicity; CAR, chimeric antigen receptor; CDK, cyclin-dependent kinase; CLL, chronic lymphocytic leukaemia; CRD, cysteine-rich domain; DFG, Asp-Phe-Gly; ECD, extracellular domain; EGFR, epidermal growth factor receptor; ER, endoplasmic reticulum; Fc, crystallizable fragment of an antibody; HER, human epidermal growth factor receptor; IL-23R, interleukin-23 receptor; JAK, Janus kinase; JH, JAK homology; KNG, kringle; KSR, kinase suppressor of RAS; mAb, monoclonal antibody; MET, hepatocyte growth factor; MLKL, mixed lineage kinase domain-like protein; PTK7, protein tyrosine kinase 7; RIPK1, serine/threonine-protein kinase 1; RYK, receptor-like tyrosine kinase; TRIB2, Tribbles homologue 2; VEGF, vascular endothelial growth factor; VEGFR, VEGF receptor; WIF, WNT-inhibitory factor.