Trends in kinase drug discovery: targets, indications and inhibitor design

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Abstract | The FDA approval of imatinib in 2001 was a breakthrough in molecularly targeted cancer therapy and heralded the emergence of kinase inhibitors as a key drug class in the oncology area and beyond. Twenty years on, this article analyses the landscape of approved and investigational therapies that target kinases and trends within it, including the most popular targets of kinase inhibitors and their expanding range of indications. There are currently 71 small-molecule kinase inhibitors (SMKIs) approved by the FDA and an additional 16 SMKIs approved by other regulatory agencies. Although oncology is still the predominant area for their application, there have been important approvals for indications such as rheumatoid arthritis, and one-third of the SMKIs in clinical development address disorders beyond oncology. Information on clinical trials of SMKIs reveals that approximately 110 novel kinases are currently being explored as targets, which together with the approximately 45 targets of approved kinase inhibitors represent only about 30% of the human kinome, indicating that there are still substantial unexplored opportunities for this drug class. We also discuss trends in kinase inhibitor design, including the development of allosteric and covalent inhibitors, bifunctional inhibitors and chemical degraders.

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Reversible protein phosphorylation mediated by kinases and phosphatases has a key role in regulating cellular functions such as cell proliferation, apoptosis, subcellular translocation, inflammation and metabolism[1,2]. The human kinome is composed of around 560 protein kinases, including approximately 500 eukaryotic protein kinases (ePKs) that are divided into eight major groups, such as tyrosine kinases, and approximately 60 atypical protein kinases, such as lipid kinases, which have a conserved kinase fold like ePKs but differ with regard to other highly conserved sequence motifs of ePKs[3,4].

Since the 1980s, protein kinases have been recognized as potential drug targets, particularly based on advances in the molecular understanding of cancer, including the discovery of oncogenes such as *SRC*[5]. However, the development of protein kinase inhibitors that bind to the ATP site was initially viewed as an unsurmountable challenge because of the high concentration of ATP in the cell, the poor understanding of the regulation of kinase activity and the conserved ATP-binding pocket. Nevertheless, the natural compound staurosporine[6] was pursued because it had potent kinase inhibitory activities, albeit with poor selectivity. By the end of the 1980s, this paved the way to identify and optimize synthetic small-molecule kinase inhibitors (SMKIs) directed at the ATP-binding site with suitable drug-like properties, selectivity and potency[7–9].

In parallel with such efforts in the 1990s, two kinase inhibitors received regulatory approval. Fasudil, an inhibitor of Rho-associated coiled-coil-containing protein kinases 1 and 2 (ROCK1 and ROCK2) was approved in Japan in 1995 for the treatment of cerebral vasospasm, although the ROCK-inhibitory activity of fasudil was not described until 1997. Sirolimus, a natural product that had a key role in the discovery of mechanistic target of rapamycin (mTOR)[10–13], became the first kinase inhibitor to reach the market in the United States in 1999, when it was approved by the FDA for the prevention of organ rejection[14], and, in retrospect, was the first allosteric kinase inhibitor. Given the history of these two drugs, imatinib (CGP 57148, STI571) — which was discovered in the labs of Ciba-Geigy in 1992 (REF.[15]) and approved by the FDA in 2001 — is perceived as a pivotal landmark in the development of kinase inhibitors. Imatinib, which potently inhibits several tyrosine kinases including BCR–ABL and the platelet-derived growth factor receptor (PDGFR), was initially approved for the treatment of BCR–ABL-driven chronic myelogenous leukaemia (CML) and subsequently for other indications such as the treatment of KIT-driven gastrointestinal tumours (GISTs) (reviewed in REF.[16]). Its remarkable clinical impact was a catalyst in the surge of activity in the field of molecularly targeted therapy in oncology, in which kinase inhibitors have continued to be prominent in the past two decades.

Box 1 | **Data collection and curation**

The agents that were identified as approved for marketing by the FDA were verified in the US registry of approved drugs at Drugs@FDA: FDA-Approved Drugs. Drugs that were approved by other regulating bodies were identified from the literature and press releases. The dataset of approved drugs that target kinases is provided in Supplementary Table 1.

The US NIH database ClinicalTrials.gov is the primary resource for clinical trial registration and contains more than 360,000 research studies within the United States and 219 countries worldwide. ClinicalTrials.gov is an agent-centric resource and hence a priori knowledge of the agents to be investigated and obtain information about is required. We used previously published drug–target interaction datasets[181,236–238] and literature reviews[21,25,239,240] as well as cross-referencing target-centric resources such as the IUPHAR Guide to Pharmacology and the OpenTargets Platform[241] and also personal communications (D. Fabbro) to build the dataset of kinase inhibitors in clinical trials. For these already-identified investigative kinase inhibitors, the mechanism of action — that is, the targeted kinases — had already been determined.

To obtain information on new kinase inhibitors entering clinical trials, the commercial resource CenterWatch was used as it posts publicly accessible weekly updates on drugs entering clinical trials as well as developments in industry pipelines. However, the molecular targets of the new agents entering clinical trials generally have to be manually verified and this information can be collated from different resources, preferably primary sources including the literature and patent information, and also other resources such as DrugBank[242], Chembl, and industry press releases and literature. Information on the clinical trials associated with each of the identified kinase inhibitors in development was obtained through ClinicalTrials.gov. Only agents with trials registered in ClinicalTrials.gov were included in the analysis and figures, although we provide information on additional agents that were found cited in the literature as currently in trials in the Supplementary information.

An advantage of our methods is that we have tried to create a unique and extensive dataset that collates agents registered in clinical trials from different sources to obtain as exhaustive a list as possible. However, owing to the dynamic nature of agents in clinical trials, as well as the time-consuming work of manually validating the mechanism of action for agents entering clinical trials, it can be difficult to maintain current drug–target resources for analysis. Hence, although kinase research is developing with many excellently curated resources, there is still difficulty in maintaining a consistent and comprehensive list of agents in clinical trials, and our curated dataset (provided in Supplementary Table 2) is likely to contain some omissions. Therefore, we consider our dataset to be a comprehensive cross-section of kinase inhibitors that have reached clinical trials.

Recent reviews on kinase inhibitors have described important aspects of kinase drug discovery, including the targeted pathways and protein families[3,17,18], FDA-only approved SMKIs[19,20], associated disease indications[21–23] or structural aspects[24,25]. However, to our knowledge there is not a recently published comprehensive analysis of kinase inhibitors and kinase targets in clinical trials, which we present here (see BOX 1 for details on the origin of the dataset and analysis, and Supplementary Tables 1 and 2 for a curated dataset). We cover trends in both FDA-approved kinase inhibitors and agents in clinical trials, which could substantially expand the proportion of the kinome that is therapeutically targeted. With such opportunities in mind, we also discuss trends and strategies in kinase inhibitor design.

Trends for approved kinase inhibitors

Since the approval of fasudil in 1995, the number of approved kinase inhibitors worldwide has increased to 98 drugs, 71 of which are SMKIs that have been approved by the FDA (as of May 2021). Remarkably, the number of approved SMKIs has more than doubled in the past 5 years with 37 FDA approvals, and SMKIs constitute approximately 15% of all novel drug approvals by the FDA from 2016 to 2021. Ten monoclonal antibodies (mAbs) that target receptor tyrosine kinases (RTKs) have also been approved by the FDA. Sixteen additional SMKIs and one mAb have been granted approval by other regulatory agencies (see details in FIG. 1 and Supplementary Table 1).

Kinase families and targets for approved drugs. The 71 FDA-approved SMKIs target 21 kinase families that constitute approximately 20% of the kinome, which corresponds with previous estimates of the coverage of protein kinase targets[24,26–28]. Kinases targeted by a drug that has been FDA-approved (which are typically considered clinically validated by industry standards) include members of families in five of the eight major ePK groups (TK, TKL, STE, CMGC and AGC), one family in the atypical protein kinase group (PIKK) and one family in the lipid kinases (PI3K; phosphatidylinositol 4,5-bisphosphate 3-kinase). Specifically, these 71 SMKIs primarily inhibit approximately 42 proteins within these groups and most of them — 49 SMKIs — predominantly target 30 proteins that are members of 15 families in the TK group (FIG. 2). Although the TK group is the most exploited kinase group, only approximately 30% of it is targeted, indicating that there is still significant room for further exploration.

Members of the HER family (HER1–4) of RTKs are the most targeted, with eight FDA-approved SMKIs and eight FDA-approved mAbs that target these proteins. The vascular endothelial growth factor receptor (VEGFR) family of RTKs has also been extensively targeted, with seven FDA-approved SMKIs and one mAb, although all of the SMKIs are non-selective inhibitors that also interact with other RTKs. Inhibition of Janus kinases (JAKs) has been another popular therapeutic strategy, and so far five SMKIs that target this family have been approved by the FDA.

Approved kinase inhibitors in oncology. There is a large body of clinical evidence that supports the driver role of kinases in cancer owing to their aberrant activation by either translocations or activating mutations[29–32]. Chromosomal translocations produce fusion proteins with abnormal localization that can be potentially oncogenic[33]. Identification and characterization of these disease drivers has facilitated the design and approval of molecularly guided cancer therapies, beginning with the pioneering example of imatinib to treat CML driven by the *BCR–ABL* translocation, which results in a protein with elevated tyrosine kinase activity[34].

The majority of FDA-approved SMKIs (61; 89%) and all the FDA-approved mAbs that target kinases (ten mAbs) have oncology indications. Following on from imatinib, four further SMKIs that target ABL have been approved so far: nilotinib, dasatinib, bosutinib and ponatinib[35,36] (see BOX 2 for details on the development of ABL inhibitors). It is noteworthy that treatment of CML with SMKIs has been shown to be long-lasting and that a substantial proportion of patients with chronic phase CML (up to 40%) did not relapse after cessation of therapy[37,38].

More than 20% (19 SMKIs and two mAbs) of the approved agents in our dataset have been approved for

Eukaryotic protein kinases (ePKs). There are eight main groups of eukaryotic protein kinases: AGC (protein kinase A, G and C); CaMK (calcium/calmodulin-dependent kinases); CMGC (cyclin-dependent kinases, MAP kinases, glycogen synthase kinases, CDC-like kinases); TK (tyrosine kinases); STE (homologues of sterile 7); CK1 (casein kinases); TKL (tyrosine kinase like); and the RGC (receptor guanylate cyclase) groups.

treatment of non-small-cell lung cancer (NSCLC), which is significant given that lung cancer is the leading cause of cancer death among both men and women, and 80–85% of lung cancers are NSCLC[39,40]. Approvals for NSCLC illustrate an important trend in kinase inhibitor development in oncology in general, in which identification of the molecular characteristics of tumours enables the development and application of first-generation SMKIs, as well as guiding further drug development to overcome the emergence of resistance[41]. In fact, next-generation inhibitors that address acquired resistance comprise up to 70% of the drugs that target seven major kinase

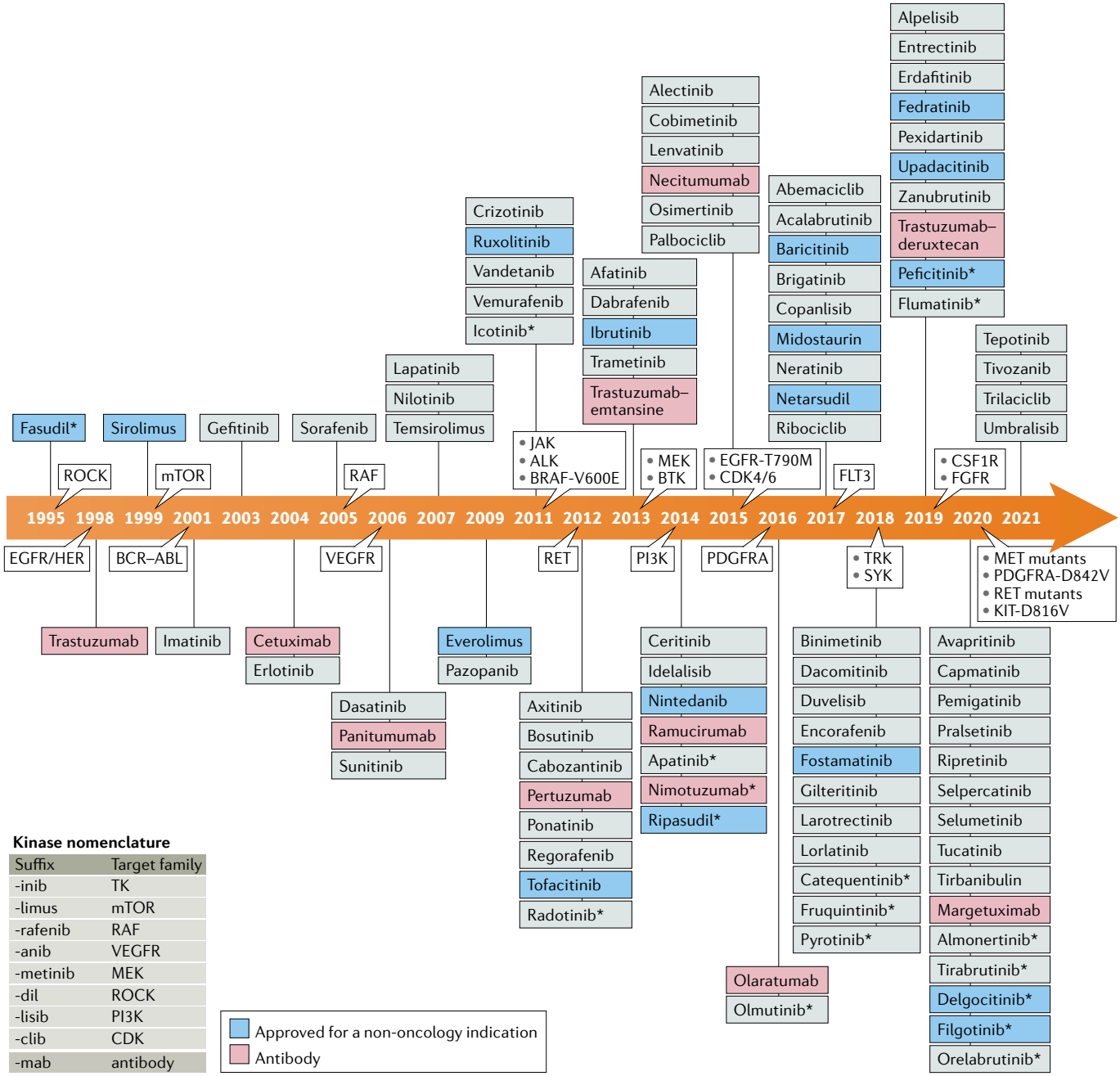


Fig. 1 | **Timeline of approved kinase inhibitors.** Beginning with the approval of fasudil in 1995, the timeline indicates the year a novel kinase family became validated (that is, the first year an agent targeting that kinase family was approved) and each of the small-molecule kinase inhibitors (SMKIs) that have been approved. Seventy-one SMKIs have been approved by the FDA; eight further SMKIs have been approved by the National Medical Products Administration (NMPA) in China; five by the Pharmaceuticals and Medical Devices Agency (PMDA) in Japan; one approved solely by the EMA, and two approved by the Ministry of Food and Drug Safety (MFDS) in South Korea (all non-FDA approvals are indicated by an asterisk). Eleven monoclonal antibodies have also been approved (indicated by a red square): ten by the FDA and one by the NMPA. Many of the kinase inhibitors have also been approved by the EMA; this timeline prioritizes approval dates from the FDA first. Several of the kinase inhibitors not approved by the FDA have been approved by more than one other regulatory agency, and the first date of approval with the corresponding regulatory agency is emphasized. Drugs that have been approved for non-oncology indications are denoted with a blue square. The suffix nomenclature for kinase inhibitors and monoclonal antibodies is provided in the key. TK, tyrosine kinase

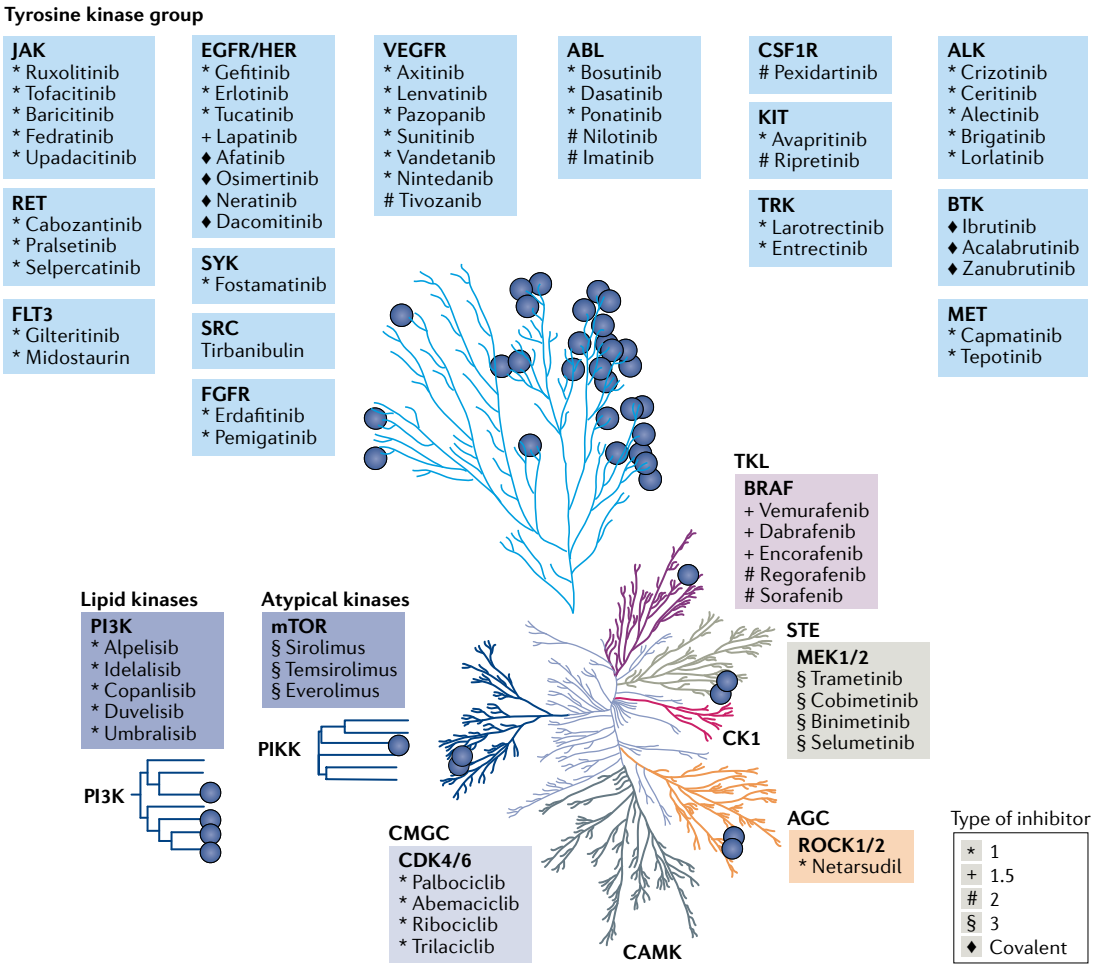


Fig. 2 | **FDA-approved kinase inhibitors mapped onto the human kinome.** The kinase targets of the 71 FDA-approved small-molecule kinase inhibitors (SMKIs) are mapped onto a phylogenetic representation of the human kinome. The primary kinase targets are identified, although many SMKIs cross-react with other kinases and in reality bind in varying degrees to other kinases. The type of kinase inhibitor is also indicated. Tirbanibulin targets the peptide substrate site of SRC.

gene families: *NTRK*, *ABL*, *ALK*, *BTK*, *FLT3*, *KIT* and *MET*. Next-generation sequencing has become the gold standard in sequencing tumours in NSCLC to detect driver mutations and facilitate comprehensive testing of multiple gene targets in a single assay. This has helped to characterize driver alterations in genes such as *EGFR*, *ALK*, *ROS1*, *NTRK* and *MET*, which are now targeted by FDA-approved drugs[42,43].

The first SMKIs to be approved for NSCLC in the early 2000s, gefitinib and erlotinib, targeted epidermal growth factor receptor (EGFR) based on its important role in tumour growth and progression[44]. However, mutation-induced drug resistance commonly occurs with SMKIs that target EGFR. In particular, T790M gatekeeper mutations arise in up to 50% of patients treated with EGFR inhibitors[45]. The single-point substitution mutation (L858R) in exon 21 and exon 19 deletions, which are often referred to as 'classical' EGFR activating mutations as they account for 85–90% of EGFR kinase domain mutations[46], are identified as sensitizing mutations associated with different clinico-pathological features[47,48]. Exon 20 frame insertions are also activating mutations; however, they are not inherently sensitive to EGFR inhibitors and have been associated with poor patient prognosis[49]. Next-generation covalent SMKIs including afatinib and dacomitinib that are active against mutations such as T790M have been approved by the FDA, and the third-generation inhibitor osimertinib, which selectively targets activating mutations as well as the T790M resistance mutation, has also been approved[50].

Rearrangements in *ALK*, which produce an abnormal ALK protein that promotes cellular proliferation and migration, are identified in approximately 5% of cases of NSCLC, and the first ALK inhibitor, crizotinib, was approved in 2011 (REF.[51]). *ROS1*, which is closely related to *ALK*, is also targeted by *ALK* inhibitors, and *ROS1* rearrangements have an incidence of approximately 1–2% in NSCLC[52]. Furthermore, acquired *ROS1* resistance mutations occur in up to 60% of crizotinib-refractory patients[53]. Second- and third-generation compounds have been developed to improve on crizotinib's therapeutic characteristics and combat resistance mutations. Four additional SMKIs that target *ALK* and/or *ROS1* translocations have since been approved by the FDA: ceritinib, alectinib, brigatinib and lorlatinib[54]. Recently, four further SMKIs that target disease-related mutations

that affect specific kinases have been approved for NSCLC: selpercatinib and pralsetinib for the treatment of *RET*-fusion-positive NSCLC; and capmatinib and tepotinib for the treatment of *MET*-mutation-positive NSCLC[55–58].

Targeting tumour angiogenesis is an important anticancer strategy, and many approved kinase inhibitors have successfully targeted the angiogenic pathways driven by VEGFR, PDGFR, KIT, fibroblast growth factor receptors (FGFRs) and MET for different indications. For example, there are five FDA-approved SMKIs approved for renal cell carcinoma that inhibit VEGFR as well as other RTKs, and also two approved rapalogue inhibitors, temsirolimus and everolimus, that target mTOR[59], which is downstream in the VEGFR signalling pathway[60]. Metastatic colorectal cancer (CRC) has also been treated via VEGFR inhibition with the FDA-approved mAb ramucirumab and the SMKI fruquintinib, which is approved in China. Medullary thyroid carcinoma has been addressed by the VEGFR inhibitors vandetanib, cabozantinib and lenvantinib, as well as the previously mentioned RET inhibitors pralsetinib and selpercatinib.

PDGFR and KIT are inhibited by imatinib, and this activity led to its FDA approval for GISTs in 2002 (REF.[61]). As with other cancers, further SMKIs have been developed to improve activity and/or address resistance to first-generation drugs, with two such agents, avapritinib and ripretinib, approved in 2020 that target particular PDGFR and KIT activation loop mutants, which are responsible for relapse of up to 85–90% of patients with GISTs[62]. Inhibition of the angiogenic FGFR pathway has more recently been validated as a therapeutic strategy, with FDA approval of erdafitinib in 2019 for the treatment of FGFR-altered advanced urothelial carcinoma and FDA approval of pemigatinib in 2020, which is the first targeted treatment for cholangiocarcinoma, an aggressive tumour characterized by *FGFR* driver mutations[63,64].

Other RTK protein families have been targeted by approved kinase inhibitors for various cancer indications. Among the most prominent are drugs that target HER2 to treat breast cancer, which include nine of the approved kinase-targeted agents in the dataset. These include the HER2-targeted mAb trastuzumab, which was approved in 1998 and provides a pioneering example of the use of an RTK-targeted agent being guided by companion diagnostics, and the very recently approved HER2-targeted mAb margetuximab, as well as four SMKIs, lapatinib, neratinib, tucatinib and pyrotinib (approved in China) that are also used to treat patients with $HER2^+$ breast cancer[65]. Two antibody–drug conjugates that target HER2, trastuzumab–emtansine and trastuzumab–deruxtecan, are also included in our dataset. Although the cytotoxic payload is a key driver of the anticancer activity of these antibody–drug conjugates rather than inhibition of the activity of HER2 alone, we include them for completeness.

Various intracellular kinases have also been targeted by approved SMKIs. Targeting CDK4 and CDK6, which have important roles in cell division, has been so successful that three SMKIs, palbociclib, ribociclib and abemaciclib, are now the standard of care in particular breast cancer indications[66]. The most recent CDK4/CDK6 inhibitor, trilaciclib, was approved in 2021 for small cell lung cancer myelopreservation therapy[67].

Box 2 | **Developing multiple generations of ABL kinase inhibitors**

Imatinib revolutionized the treatment of chronic myelogenous leukaemia (CML) and provided proof for the concept that small molecules that specifically target molecular drivers of cancer, such as the mutant BCR–ABL kinase in CML, can provide effective therapy. Various efforts have been made to develop second- and third-generation ABL inhibitors that could overcome the intrinsic clinical resistance of CML to imatinib due to mutations in the kinase domain of ABL (including the gatekeeper mutation T315I)[109,243,244]. One approach has been structure-based drug design, enabled by the elucidation of the imatinib–ABL co-crystal structure, to improve the molecular recognition of inhibitors in the ATP-binding site, which resulted in the approval of nilotinib and ponatinib[245–249]. A second approach in which compounds were screened against both wild-type and mutant BCR–ABL led to the approval of dasatinib and bosutinib as broad-spectrum BCR–ABL inhibitors. However, except for ponatinib, none of these second-generation ATP-binding-site-directed BCR–ABL inhibitors was able to inhibit the gatekeeper T315I mutant.

A phenotypic screen using *BCR–ABL*-transformed cell lines resulted in the discovery of GNF-2, which seemed not to be directed to the ATP-binding site of ABL, as it was unable to inhibit enzymatic ABL kinase activity when the kinase domain only of ABL was used[250]. The structure of ABL with its regulatory SH2 and SH3 domains lacking the last exon showed that the N terminus of ABL was myristoylated and that myristate binding into a cylindrical pocket located at the C terminus of the kinase domain (the myr pocket)[244,245] led to an auto-inhibited conformation[136,137]. However, when BCR is fused to the N terminus of ABL, the N-terminal myristoyl is lost, which results in the constitutive ABL kinase activity of BCR–ABL[136,137]. The binding of GNF-2 into the myr pocket was demonstrated by X-ray crystallography and NMR[136]. In addition, binding of GNF-2 to the myr pocket was disrupted by point mutations that also conferred resistance to the ability of GNF-2 to inhibit cellular BCR–ABL activity[251,252]. Further structural and experimental analyses indicated that GNF-2 could allosterically inhibit BCR–ABL kinase activity, even with the recalcitrant T315I gatekeeper mutation in ABL[245]. This led to a search for second-generation myr-pocket binders (third-generation ABL inhibitors), resulting in the discovery of ABL001 (asciminib; also known as STAMP (specifically targeting the ABL myristoyl pocket)), which can induce an auto-inhibited conformation of even the ABL-T315I mutant[245,251].

The improved potency of asciminib against wild-type ABL and ABL-T315I translated into a high degree of synergistic activity with ATP-site-directed inhibitors against cells transformed with BCR–ABL-T315I[136]. This inhibitor is among the most potent and specific ABL kinase inhibitors known to date. Asciminib has been tested in clinical trials involving heavily pretreated patients with CML who had resistance to or unacceptable side effects from other kinase inhibitors, including patients in whom ponatinib had failed and those with a T315I mutation. A phase III trial met its primary end point, showing that treatment with asciminib resulted in a higher rate of major molecular responses than bosutinib[253], and a regulatory submission for asciminib is expected in 2021.

The development of BCR–ABL kinase inhibitors illustrates some general lessons for kinase drug discovery. First, allosteric kinase inhibitors can be very selective; asciminib is one of the most selective kinase inhibitors among the synthetic SMKIs. Second, biochemical assays to search for kinase inhibitors are best done with native and full-length proteins if possible to have access to diverse regulatory interactions. Third, understanding the mechanism of action of kinase inhibitors requires time and labour-intensive commitment to medicinal chemistry, pharmacology, biochemistry, biophysics, and cell and structural biology. It took 13 years from the approval of imatinib to the phase I studies with asciminib. Finally, it is important to understand the disease that is being treated by kinase inhibitor drugs. Imatinib and the other inhibitors that target ABL for the treatment of CML still stand out in terms of their unprecedented efficacy compared with many kinase inhibitors developed for other cancers in the past two decades. This may reflect that imatinib and the other BCR–ABL inhibitors are conceptually closer to preventing cancer, namely the acute phase of CML (blast crisis) rather than treating a cancer, which may often be at an advanced stage. Indeed, the chronic phase of CML, in particular the early phase, could be viewed as a myeloproliferative disorder rather than a cancer. Many myeloproliferative disorders are driven by tyrosine kinases, for example, polycythemia vera (JAK2), chronic myelomonocytic leukaemia (PDGFR) and hypereosinophilic syndrome (PDGFR), although they rarely turn into cancer, unlike CML.

Metastatic melanoma is a challenging disease to treat, and the commonly observed *BRAF*^{V600E} mutation makes this cancer particularly aggressive. Six SMKIs have been approved for this indication: three agents are BRAF inhibitors (vemurafenib, dabrafenib and encorafenib) and three are MEK inhibitors (trametinib, cobimetinib and binimetinib)[68]. Furthermore, the MEK and BRAF combination regimen of dabrafenib plus trametinib was also approved by the FDA in 2018 (REF.[69]). Additionally, evidence indicates that combinations of targeted therapy with SMKIs, and particularly MEK and BRAF inhibitors, plus immunotherapy can achieve durable responses with better therapeutic effectiveness[70,71].

The PI3K–AKT–mTOR pathway is involved in cell survival, metabolism and regulation of cell growth. The dysregulation of this pathway in many cancers[23] has led to extensive efforts to target its components. Such efforts have led to the only approved kinase inhibitors that target lipid kinases, beginning with FDA approval of idelalisib, a first-in-class SMKI that targets PI3Kδ, for the treatment of various lymphomas in 2014 (REFS[72,73]). Since then, the dual PI3Kα/β inhibitor copanlisib was approved in 2017 for follicular lymphoma[74], the dual PI3Kδ/γ inhibitor duvelisib was approved in 2018 for several lymphomas[75] and the PI3Kα-selective inhibitor alpelisib was approved in 2019 for the treatment of breast cancer[76]. Three SMKIs have been approved that target mTOR (see above), including sirolimus — one of the earliest kinase inhibitors — which has been approved for a variety of indications, both oncological and non-oncological.

Substantial progress has been achieved by targeting kinases for the treatment of other haematological malignancies. Notably, mantle cell lymphoma and chronic lymphocytic leukaemia are being addressed by three FDA-approved Bruton tyrosine kinase (BTK) inhibitors[25,77]: ibrutinib, acalabrutinib and zanubrutinib. Significantly, all three drugs act covalently, and the success of ibrutinib had an important role in catalysing a re-evaluation of covalent inhibition as a drug design strategy (see below), together with afatinib (see above).

Finally, kinase inhibitors are also at the forefront of pioneering efforts to develop anticancer drugs based on the molecular characteristics of the tumour, instead of the tissue of origin[78]. Three decades after the discovery of NTRK1 as a component of an oncogenic fusion gene product in CRC[79] and the subsequent discovery of *NTRK2* and *NTRK3*, the pioneering pan-TRK inhibitor larotrectinib became only the second tumour-agnostic therapy to enter the market (after pembrolizumab) following its FDA approval in 2018 for the treatment of patients with solid tumours that have an *NTRK* gene fusion[80]. Since then, it has been joined by the multikinase inhibitor entrectinib, which is also approved for patients with solid tumours that have an *NTRK* gene fusion[81]. Other kinase inhibitors are also being investigated in tumour-agnostic development programmes, including the approved RET inhibitors pralsetinib and selpercatinib[78].

Approved kinase inhibitors beyond oncology. Many kinases are involved in the regulation of immune responses. For instance, JAKs are the key components for T helper cell (T_H1, T_H2 and T_H17) immune responses as they are secondary messengers for many cytokines, including interferons and interleukins[82,83]. Eleven SMKIs — more than 13% of the approved SMKIs — have been approved for immune system-related indications, mostly autoimmune and inflammatory disorders.

The first kinase inhibitor to be approved outside oncology was ruxolitinib, an inhibitor of JAK1 and JAK2 that was approved by the FDA for the treatment of patients with intermediate or high-risk myelofibrosis in 2011, following the recognition that activating mutations in *JAK2* caused a group of myeloproliferative neoplasms[84]. A JAK2 inhibitor, fedratinib, was also approved for such diseases in 2019.

Rheumatoid arthritis is the autoimmune disease with the most approved SMKIs (five overall, and three approved by the FDA). All five SMKIs selectively target isoforms of JAK, and include the first SMKI to be approved for rheumatoid arthritis — tofacitinib in 2012 — followed by baricitinib, upadacitinib, peficitinib and filgotinib. Peficitinib predominantly inhibits JAK3 and was approved by the Pharmaceuticals and Medical Devices Agency (PMDA) in Japan in 2019. Filgotinib is a selective JAK1 inhibitor that has received approval by the EMA and PMDA; however, the FDA has postponed a decision on the new drug application for it for safety reasons (see Related links). JAK kinase inhibitors are also being investigated for other immune disorders, and delgocitinib, a pan-JAK inhibitor that was approved in 2020 by the PMDA, is the first kinase inhibitor approved for the skin disorder atopic dermatitis.

The kinase SYK, which is involved in innate and adaptive immune responses, is implicated in the development of allergic and autoimmune diseases, as well as in haematological malignancies[85–87]. Fostamitinib, a first-in-class SYK inhibitor, was approved by the FDA in 2018 for chronic immune thrombocytopenia owing to its ability to reduce antibody-mediated destruction of platelets[88]. Rejection prophylaxis for organ transplantation and graft-versus-host disease are also immune-related issues that are amenable to kinase inhibition. Two agents have been approved for each of these disorders: ibrutinib and ruxolitinib.

Finally, several SMKIs have been approved for diseases beyond oncology and immunology. These include the ROCK1 and ROCK2 inhibitor fasudil for cerebral vasospasm (mentioned above), the mTOR inhibitor everolimus for tuberous sclerosis complex-associated partial-onset seizures[89], the VEGFR inhibitor nintedanib for idiopathic pulmonary fibrosis[90], the FLT3 inhibitor midostaurin for advanced systemic mastocytosis[91] and another ROCK1/ROCK2 inhibitor, netarsudil, for glaucoma and ocular hypertension[92].

Classes of agents. Nearly all of the FDA-approved SMKIs are directed towards the kinase ATP-binding site (63 SMKIs), with the exception of three rapalogues, four MEK inhibitors and the dual SRC and tubulin polymerization inhibitor tirbanibulin, which targets the peptide substrate site[93] (see below for further discussion of binding modes). The conservation of the ATP-binding site in the human kinome means that 'ATP mimetics' often

cross-react with many other different kinases, resulting in compounds with promiscuous profiles. Promiscuous compounds such as dasatinib[94] or sunitinib[95,96] have been termed multikinase inhibitors and can have some toxicological liabilities[97]. However, this cross-reactivity can also be potentially advantageous. For instance, the activity of imatinib against multiple kinases has enabled its application in multiple indications, such as CML driven by the *BCR–ABL* translocation, GISTs driven by KIT and hypereosinophilic syndrome driven by PDGFRα deregulation. Inhibiting multiple nodes in cellular networks by blocking two or more kinases can also have synergistic effects that might be clinically useful; for example, targeting the BRAF and MEK pathways with the previously mentioned combination of dabrafenib plus trametinib[98].

The cell surface location of RTKs has been successfully exploited by ten FDA-approved mAbs for the treatment of breast cancer, CRC, NSCLC, soft tissue sarcoma and hepatocellular cancer. The mechanism of action of such agents is more complex than inhibition of kinase activity by SMKIs, and could include blockade of ligand binding (thereby preventing kinase activation), promoting receptor internalization and antibody-dependent cellular cytotoxicity[99].

Trends in kinase inhibitor design

The identification of SMKIs has been advanced substantially by understanding of the structures of their binding sites. More than 5,500 catalytic domain structures have been published so far, covering approximately 300 kinases from 104 families. In this section, we overview the key features relevant to structure-based drug design and trends in the development of SMKIs of different types.

Structural features of the kinase catalytic domain. The architecture of the kinase fold was first described in 1991 by Knighton et al.[100,101], who reported the structure of protein kinase A (PKA) in complex with ATP–Mg^{2+} and an inhibitory peptide. This structure revealed a bilobal arrangement including an N-terminal smaller lobe containing a five-stranded antiparallel β-sheet (β1–β5), the conserved regulatory αC helix, two additional helical segments, as well as a larger C-terminal lobe of mostly α-helices (FIG. 3a).

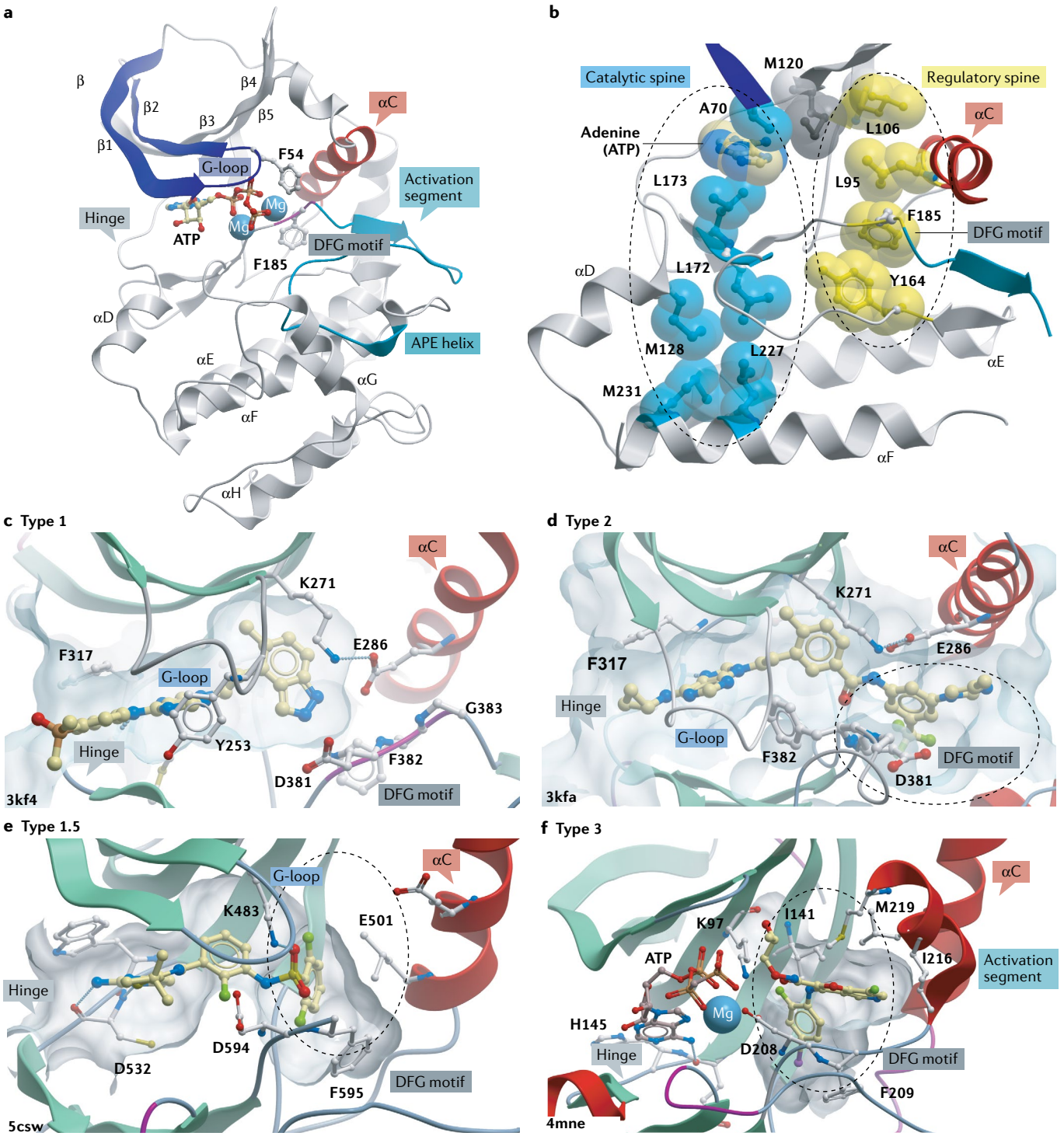
The ATP cofactor was found sandwiched between the two lobes and forming hydrogen bonds between the adenine ring and the hinge region, which established a backbone connecting the lobes. These hydrogen bonds represent important anchor points for the design of ATP mimetic inhibitors and define the basic kinase inhibitor scaffolds. ATP is also coordinated by the glycine-rich loop (G-loop, also called phosphate-binding loop (P-loop)). This is a highly flexible region that is present in the β-sheet structures β1 and β2 with the consensus sequence motif GXGXφG, where φ represents a hydrophobic residue. A second key sequence motif, (V)AxK, is located in β3.

A structural hallmark of active kinases is a conserved salt bridge formed between the lysine residue in the (V)AxK motif and a conserved glutamate located in αC positioning αC ('αC-in' conformation). The αC helix has an important regulatory role, as the (V)AxK salt bridge contributes to the coordination of the phosphate groups of ATP. In inactive kinases, the αC helix dislodges from its position in the active state moving outwards (αC-out) and it often also rotates the central conserved glutamate away from the ATP-binding site.

The C terminus of the αC helix in the αC-in state is additionally stabilized by interaction with the conserved tripeptide Asp-Phe-Gly motif DFG, which also anchors the N terminus of the activation segment. In its active 'DFG-in' conformation, the DFG aspartate residue points towards the phosphate moieties of ATP, coordinating a catalytically important Mg^{2+} ion. In inactive kinases, the DFG is mobile, dislodging the phenylalanine residue. This so-called 'DFG-out' conformation opens a deep, mainly hydrophobic binding pocket[102] (see below). The DFG-out movement also results in unstructuring of the activation segment, a mobile structural element of variable length and sequence that harbours the DFG motif, the activation loop (A-loop) and ends with the helical APE (Ala-Pro-Glu) motif. The A-loop also contains phosphorylation sites that regulate kinase activation of kinases that are not constitutively active, by either autophosphorylation or transphosphorylation[103]. Finally, the catalytic loop harbours a highly conserved Y/HRD (Tyr/His-Arg-Asp) motif with the strictly conserved aspartate, which is required for catalysis.

The 3D structure of ePKs is highly conserved, including a network of structurally important hydrophobic residues[104]. These residues form two spines that in the active state connect and properly align both kinase lobes and position key sequence motifs for catalysis[104,105]. One spine is called the catalytic spine (C-spine), which is complemented by the adenine ring system of the cofactor ATP, and connects structural elements at the hinge side of the kinase domain. A second hydrophobic spine called the regulatory spine (R-spine) senses the proper alignment of the β4 sheet, αC, the DFG motif and the lower lobe αE helix when in the active state (FIG. 3b). In inactive states, the displacement of R-spine residues causes the R-spine to be broken. The functional importance of spine residues has been validated experimentally by mutagenesis studies that demonstrated that hydrophobic contact by spine residues is required for proper catalytic function[106]. Owing to their proximity to the ATP-binding site, many kinase inhibitors interact with spine residues or even intercalate or alter spine interactions.

A residue called the gatekeeper bridges the C-spine and the R-spine (M120 in PKA) and is located at the N terminus of the hinge region. The importance of the gatekeeper residue for drug development was recognized early[107]. The presence of a small gatekeeper residue such as threonine, although not common, can enable the design of inhibitors that are selective over other kinases because it makes the back pocket accessible to small molecules; this means that inhibitors that bind to this pocket are prevented from binding to other kinases that have large hydrophobic residues in the gatekeeper position[108]. Glycine residues are not present in the gatekeeper position in human kinases, which led to the design of bulky ATP analogues that specifically target kinase gatekeeper



mutants. However, gatekeeper mutations that shift to bulkier residues that sterically exclude inhibitors are a common cause of kinase drug resistance[109].

Comprehensive selectivity testing. The development of large panels of kinase assays for screening has enabled comprehensive assessment of inhibitor selectivity in and outside cells[110]. The assays are either enzyme kinetic assays, stability-based assays[111] or displacement assays using recombinant proteins[112] or cellular extracts in combination with mass spectrometry[113,114]. Recently, assay systems have also been established that allow studies on inhibitor binding to full-length kinases and selectivity in live cells[115,116]. In particular, besides the well-established potency criteria, kinetic aspects of ligand binding have also emerged as important parameters for ligand optimization[117,118].

Strategies for rational inhibitor design. The highly dynamic nature of protein kinases allows for the design of inhibitors that recognize the active or diverse inactive conformations. The inactive state of kinases is

◀ Fig. 3 | **Structural features of the kinase catalytic domain and inhibitor binding modes. a** | Structural overview of the kinase catalytic domain, based on the structure of protein kinase A (PKA) in ribbon representation[235]. The main secondary structure elements are labelled in parts **a** and **b**. The ATP cofactor is shown in ball and stick representation. Mobile structural elements that regulate kinase activity including the glycine-rich loop (G-loop; blue), the activation segment (turquoise) and αC helix (red) are highlighted. Two Mg^{2+} ions are shown as solid spheres. **b** | Aligned catalytic (blue) and regulatory (yellow) spine residues in the PKA active state. The gatekeeper residue (M120, grey) bridges the two spines. The adenine ring of ATP bridges N-terminal and C-terminal lobe catalytic spine residues. The secondary structure elements are coloured in light green for sheets and red for helices in parts **c**–**f**. **c** | Details of the type 1 binding mode in ABL kinase. The binding site boundaries are shown as a transparent surface. Key residues are shown in ball and stick representation. Hydrogen bonds are shown as dotted lines. **d** | Details of the type 2 binding mode in ABL kinase. Labelling of residues and structural elements is identical to panel **c**. The large pocket created by the Asp-Phe-Gly motif (DFG)-out movement that is characteristic of this binding mode is highlighted by a dashed circle. **e** | Details of the type 1.5 binding mode in BRAF kinase complexed with dabrafenib. Binding of the inhibitor breaks the regulatory spine by inducing an αC-out movement. The salt bridge between αC E501 and K483 is not present in this inactive conformation. **f** | Example of a type 3 inhibitor binding mode in the kinase MEK1. The inhibitor occupies a pocket (dashed circle) created by an αC-out movement and an inactive helical conformation of the activation segment. The cofactor ATP–Mg^{2+} shown in ball and stick representation binds to the ATP-binding site and the DFG motif is in a DFG-in conformation. APE, Ala-Pro-Glu motif.

particularly structurally diverse and has been extensively explored in drug development. More than 80 possible ligand-binding sites that are present in the kinase catalytic domain have been catalogued and described[3,119]. A detailed description of this area is beyond the scope of this Review and we restrict our description to the main binding modes that are now well-established in kinase drug discovery (for detailed reviews, see REFS[25,120]).

Type 1 inhibitors target the kinase active state (FIG. 3c). The central purine moiety of the inhibitor mimics hinge-binding hydrogen bonds of ATP, and both αC and the DFG motif are in their active 'in' position. However, in this structure, the G-loop is not in an active conformation forming an aromatic stacking interaction with the purine ring and Y253. Aromatic amino acids are frequently found at the tip of the G-loop. In the ATP-bound active state, these residues are oriented away from the ATP site. Interestingly, so-called 'folded P-loop conformations' have been observed in many kinase inhibitor complexes, and this conformation has been associated with favourable inhibitor selectivity[121].

Type 2 inhibitors bind to an inactive kinase state, which is characterized by the DFG-out conformation (FIG. 3d). As shown in the figure, the outward movement of the DFG motif creates a large hydrophobic pocket that is targeted by the trifluoromethylphenyl moiety of this inhibitor. The αC helix maintains its active state αC-in position, as indicated by the formation of the canonical salt bridge between (V)AxK K271 and the conserved αC glutamate E286, while the G-loop maintains its folded conformation.

Many intermediate binding modes between those of canonical type 1 and type 2 inhibitors exist, and some authors have introduced type 1.5 binding modes. The definition of type 1.5 inhibitors in the kinase literature is not clear, however. A stringent definition could be an inhibitor that disrupts the R-spine, but the term has also been used to describe inhibitors for which binding results in any type of structural deviation from the ideal active conformation, including abnormal G-loop conformations and auto-inhibitory conformations of the activation segment[122]. As many of these structural elements are highly mobile, and their conformations may depend on crystal packing, we consider it more useful to limit this binding mode type to inhibitors that disrupt the R-spine without targeting the canonical type 2-specific DFG-out pocket (FIG. 3e). In the BRAF–dabrafenib complex, the αC helix is moved outwards, resulting in a broken R-spine and a broken (V)AxK (K483) αC glutamate (E591) salt bridge, while the DFG motif remains in a DFG-in position[123].

Non-canonical binding modes represent attractive design strategies, as these compounds are often highly selective compared with canonical type 1 and type 2 inhibitors and have prolonged target residence times. For instance, the EGFR inhibitor lapatinib binds to a DFG-in conformation, but it induces large structural rearrangements that explain its high selectivity for EGFR[124]. Similarly, the protein kinase R (PKR)-like endoplasmic reticulum kinase (PERK) inhibitor GSK2606414 and the MET inhibitor SGX523 target an inactive conformation of the activation segment. The ERK1/ERK2 inhibitor SCH772984 specifically interacts with a unique binding pocket created by an αC-out and a folded P-loop conformation[125–128]. The Kinase–Ligand Interaction Fingerprints and Structures (KLIFS) database provides a valuable resource for studies on ligand interactions and the distribution of diverse binding modes across the kinome[129].

Recent studies have also examined the role of water in ligand design. Early models considered the displacement of water based only on the simplistic view of entropic gain when ligands displace binding-site waters. More refined computational tools now characterize each water molecule seen in structural models in detail to evaluate the role of water in ligand potency, conformational change and ligand off-rates[130,131].

Trends in the chemistry of approved kinase inhibitors. The ATP-binding site is the main site targeted by approved SMKIs, accounting for 63 of the 71 FDA-approved drugs. Similar to the ATP adenine ring, ATP mimetic inhibitors are anchored to the kinase hinge backbone by hydrogen bonds. Several scaffolds have been developed that act as hinge binders (FIG. 4). Fused six-membered ring systems, such as (iso)quinoline and quinazoline, which was first established in the development of fasudil and subsequently gefitinib and erlotinib, have been the most frequently used ATP mimetics, with 18 approved SMKIs. The aminopyrimidine and closely related aminopyridine moiety, which was used in imatinib, have also remained frequently used hinge binders, with 17 approved SMKIs.

The third group of hinge binder moieties are fused five- and six-membered ring systems such as pyrrolopyrimidines and pyrrolopyrides, purines, oxoindoles and pyrrolo or imidazo triazines. Structurally, pyrazolo aminopyrimidines and imidazo aminopyrazines are also related to this class of hinge binders, but based on their hinge-binding modes, they should be considered as derivatives of aminopyrimidines. Non-aromatic

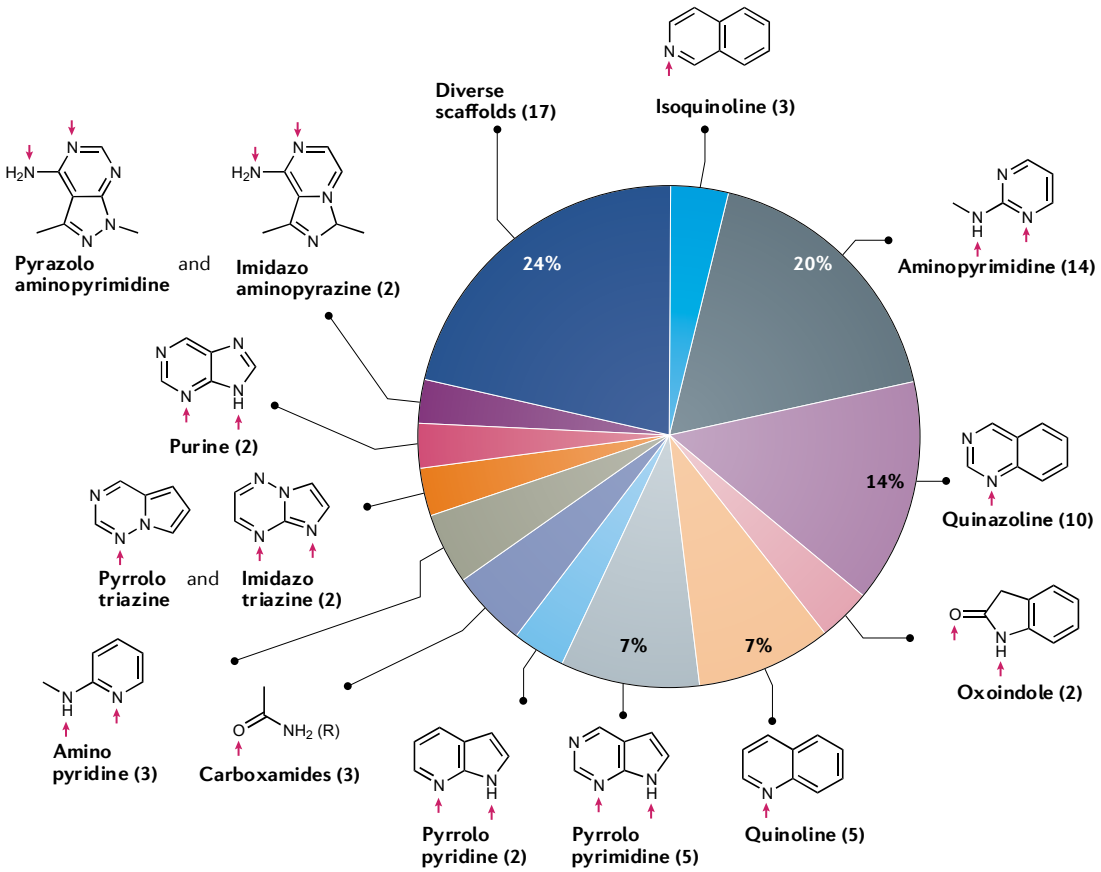


Fig. 4 | **Chemical scaffolds used in approved kinase inhibitors.** Chemical structures of the major hinge-binding scaffolds used in approved kinase inhibitors. Shown is the distribution of hinge-binding scaffolds for all ATP mimetic inhibitors (centre) as well as the structures of the most frequently used moieties. The number of approved drugs using each major scaffold is provided in brackets and each chemotype has been linked to the central pie chart by colour and numbering. Hydrogen bond donors and acceptors are indicated by red arrows.

hinge-binding motifs are represented by carboxamides. A total of 17 SMKIs harbour diverse hinge-binding scaffolds, but the narrow chemical space explored in the development of two-thirds of the approved SMKIs is astounding.

Allosteric inhibitors. Most signalling kinases are expressed in an inactive state that is activated by many different mechanisms, such as structural changes induced by post-translational modification or through interaction with scaffolding proteins or flanking regulatory domains. Thus, the key role of kinases regulating cellular signalling requires a high degree of catalytic domain plasticity. This characteristic has been widely explored through the development of allosteric inhibitors that prevent kinase activation.

Allosteric kinase inhibitors differ in the activation mechanisms that they prevent and they have therefore been classified only by their location of binding. Type 3 inhibitors bind adjacent to the ATP-binding site in a pocket induced by an αC-out conformation and in some cases by an inactive conformation of the A-loop (FIG. 3f). All four clinically approved MEK1/MEK2 inhibitors are examples of type 3 inhibitors that bind to the allosteric back pocket. The DFG is in an active 'in' conformation, allowing simultaneous binding of the type 3 inhibitor and ATP. Some type 3 inhibitors also form polar interactions with the phosphate backbone of ATP, resulting in an ATP-uncompetitive mode of action. However, an increasing number of type 3 inhibitors bind to the canonical back pocket but stabilize a DFG-out conformation. Examples include allosteric inhibitors that target FAK[132], TRKA[133], PAK1 (REF.[134]) and IGF1R[135]. Remarkably, these inhibitors show isoform selectivity against closely related family members.

Type 4 inhibitors bind to allosteric sites that are distantly located from the ATP-binding site (schematic representations of binding types in FIG. 5). An example of a type 4 inhibitor is the ABL inhibitor GNF-2, which binds to an induced pocket in the C-terminal kinase lobe that also serves as a binding site for lipids such as myristate[136] (BOX 2). This type 4 inhibitor makes use of the unique ABL activation mechanism: in inactive ABL kinases, a myristoylated residue at the N terminus of ABL binds to the C-lobe binding pocket, which results in the stabilization of a closed inactive conformation[137]. In the oncogenic fusion protein BCR–ABL, this mechanism of inactivation is lost owing to the presence of the N-terminal fusion partner BCR, which results in constitutively active ABL. GNF-2 and the investigational type 4 inhibitor asciminib (ABL001) mimic the binding of myristate and induce an inactive state similar to that

observed for the inactive wild-type protein. Interestingly, combining myristate mimetic ABL type 4 inhibitors with conventional ATP-competitive drugs could reduce the risk of development of drug-resistant mutations[138].

Several other type 4 inhibitors have entered clinical testing, including the allosteric AKT1 inhibitor MK-2206 (REFS[139,140]), which binds at the interface between the AKT catalytic domain and the pleckstrin homology (PH) domain. The kinase is locked in an inactive conformation when it is targeted and bound by MK-2206. This also prevents recruitment of AKT1 to the plasma membrane, which is a re-localization event that is required for AKT1 activation. A wide range of preclinical allosteric inhibitors that target diverse binding sites such as the PDK1-interacting fragment (PIF) pocket[141] have now been reported[120]. Allosteric inhibitors also allow selective targeting of mutations, as demonstrated by EAI045, which targets the EGFR resistance mutants EGFR-L858R/T790M and EGFR-L858R/T790M/C797S while largely sparing wild-type EGFR[142].

Allosteric targeting of protein kinases offers the opportunity to mimic natural regulatory mechanisms in a highly specific manner. The therapeutic potential of these modulators of kinase activity is enormous and the kinase field has only started to explore this targeting strategy in a clinical setting.

Covalent inhibitors. Several covalent kinase inhibitors have been approved since the pioneering approvals of the covalent EGFR inhibitor afatinib[143] and the BTK inhibitor ibrutinib by the FDA in 2013 (see above). Seven additional SMKIs have been recently approved that target either EGFR mutants or BTK (Supplementary Table 1). All currently approved covalent kinase inhibitors except acalabrutinib use an acrylamide group as the electrophile for covalent bond formation (FIG. 5b). Substitution of the alpha position in an acrylamide by an electron-withdrawing group such as a cyano group allows for the design of reversible covalent inhibitors[144]. This has been explored in the development of several highly selective tool molecules with prolonged target residence times[145,146]. The potential for using covalent targeting for the design of highly selective kinase inhibitors is huge and this design strategy has still not been widely explored[147,148].

Macrocycles. Macrocyclization has emerged as a recent strategy to improve inhibitor potency and selectivity as well as pharmacokinetic properties. Lorlatinib, for instance, has been developed on the basis of its non-cyclized template, crizotinib[149]. Macrocyclization locked the bioactive conformation of crizotinib, which resulted in markedly improved potency against ALK and ROS, as well as outstanding central nervous system penetration[150]. In addition, lorlatinib is effective against resistance mutations for first- and second-generation ALK inhibitors[151], and it has improved pharmacological properties compared with its parent compound. Another interesting example of successful macrocyclization is the EGFR inhibitor BI-4020, which has been designed to target tertiary EGFR resistance mutations, such as EGFR-L858R, EGFR-T790M and EGFR-C797S that emerge in NSCLC[152].

Bifunctional inhibitors. A plethora of additional classes of inhibitor have emerged recently that comprise bisubstrate and bivalent inhibitors, as well as molecular glues that induce new protein interactions by binding to protein interfaces (FIG. 5b). These agents link inhibitors that interact with different binding sites; for example, ATP mimetic inhibitors with SH2 domain ligands or substrate-competitive inhibitors[153–156]. Such inhibitors have been referred to as type 5 inhibitors in some publications. However, since these inhibitors are composite inhibitors that combine type 1 inhibitors via a chemical linker with a compound that targets a remote secondary binding site, we see no conceptual advantage to introducing a new name for this diverse inhibitor class. Their large molecular weight makes it challenging to develop these inhibitors clinically, but the increase in selectivity usually observed for these bidentate inhibitors may provide interesting chemical tools for target validation and functional studies.

Chemical degraders such as proteolysis-targeting chimeras (PROTACs) also fall into the class of bidentate inhibitors. PROTACs have received a lot of attention recently owing to their ability to catalytically degrade a target molecule, often with increased isoform and mutant selectivity[157,158]. This can be achieved by linking a conventional kinase inhibitor to a ligand that binds to an E3 ligase. Protein kinases seem to be highly amenable to degradation. A recent study reported PROTAC lead compounds for approximately 200 ePKs using promiscuous kinase inhibitors[159]. Selective PROTACs have already been developed for major kinase drug targets, including BRAF[160], EGFR-L858R/T790M oncogenic mutants[161], JAK[162], BTK[163], ALK[164], AKT[165], CDK6 (REFS[166,167]), CDK9 (REF.[168]), TBK1 (REF.[169]), Aurora kinase A[170], TRKA[171], FAK[172] and others. As for bidentate inhibitors in general, the pharmacological challenges to develop these molecules for clinical applications are greater than for conventional small molecules, but the first compounds have now entered clinical testing[173]. In the near future it is likely that more PROTACs will enter clinical development and it will be exciting to see how PROTACs perform in clinical studies compared with conventional inhibitors.

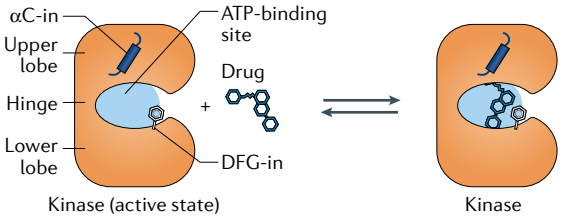
An attractive variant of PROTACs are molecular glues that bind to the interface of two proteins, resulting in the formation of a complex. In the kinase field, molecular glues that modulate kinase function were discovered early on. The macrolide rapamycin and its related rapalogues allosterically inhibit mTOR by inducing the formation of a ternary complex between FKBP12 (FK506 (tacrolimus)-binding protein 12), rapamycin and mTOR[174]. Molecular glues can be used in the context of the ubiquitin system as selective degraders by recruiting novel substrates (neosubstrates) to E3 ubiquitin ligases. Since the identification of the E3 ligase cereblon as a target of thalidomide[175], several transcription factors have been identified as neosubstrates including the C2H2 zinc finger protein SALL4, a key factor in the teratogenicity of thalidomide[176]. This explains the teratogenic mechanism of thalidomide as well as its immunomodulatory

Molecular glues
In the context of targeted protein degradation, molecular glues are small molecules that induce association of ubiquitin ligases with their target via monovalent interactions. However, designing a small molecule that can bind to both a ubiquitin ligase and a kinase in this way is highly challenging, and so molecular glues are most often identified by screening compound libraries.

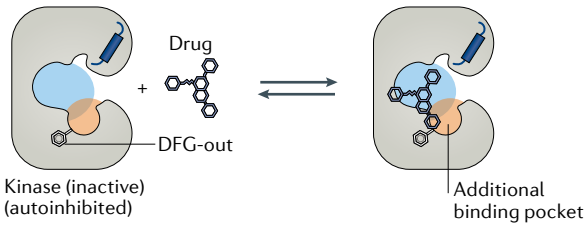
Proteolysis-targeting chimeras
(PROTACs). Bivalent macromolecules composed of a flexible linker that is capped with protein-binding moieties designed to bring ubiquitin ligases and a target such as the kinase of interest into close proximity to promote its degradation. Although this strategy could enable more specific targeting of a given kinase, the large molecular size of PROTACs may pose challenges related to drug characteristics such as oral bioavailability.

a Kinase inhibition modes

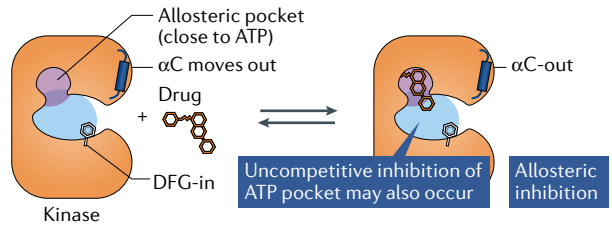
Type 1 inhibitors (ATP mimic)



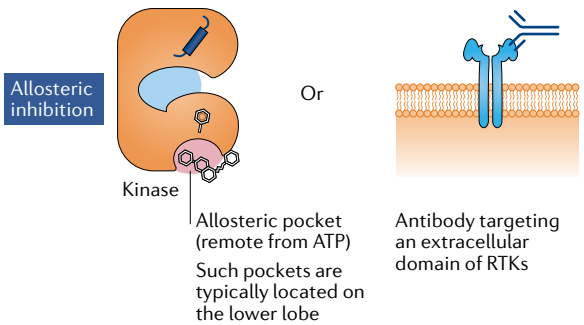
Type 2 inhibitors (ATP mimic)



Type 3 inhibitors (allosteric close to ATP)

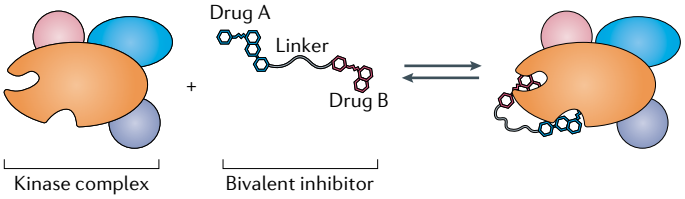


Type 4 inhibitors (allosteric remote from ATP)

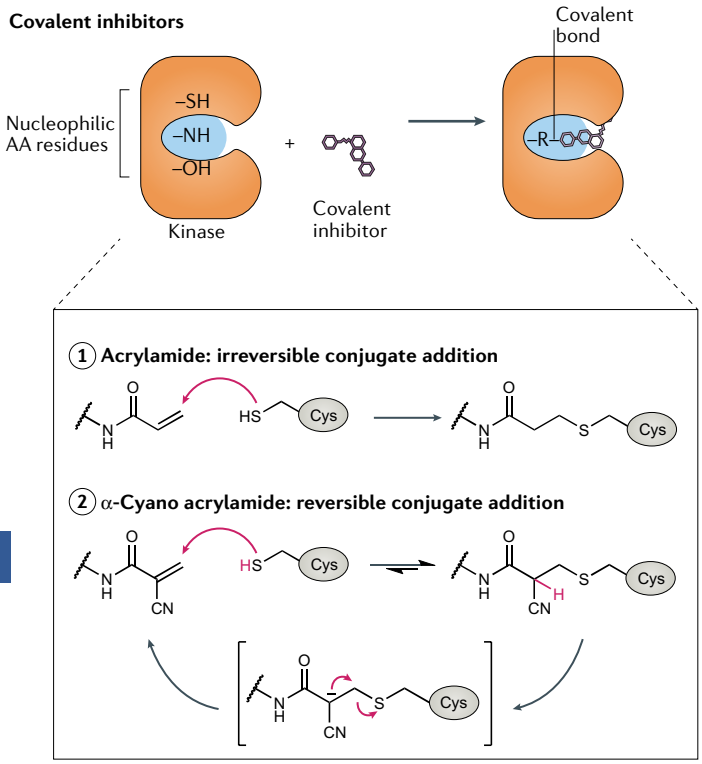


b Additional approaches for kinase targeting

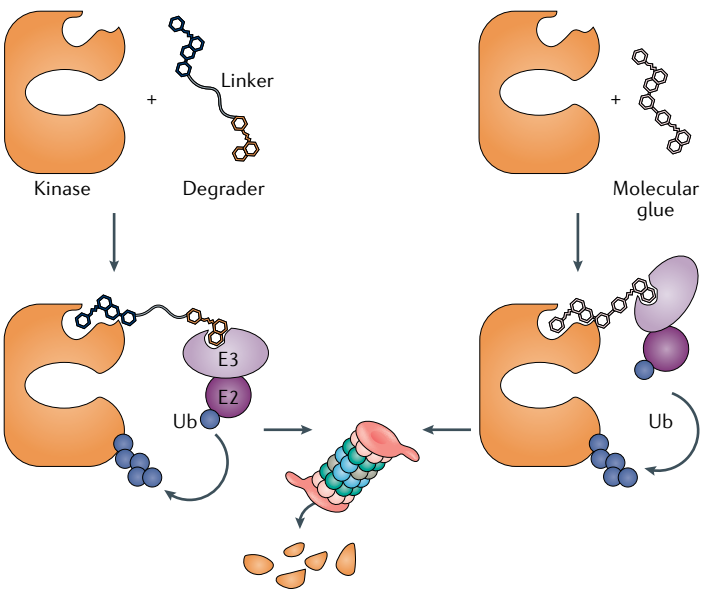
Bivalent inhibitors



Covalent inhibitors



Degraders and molecular glues



◀ Fig. 5 | **Schematic representation of kinase inhibitor binding modes. a** | Kinase inhibition modes. There are four primary types of kinase inhibitor. The colour of kinases indicates their state (active: orange; inactive: grey). Type 1 inhibitors inhibit kinases at the ATP site with the Asp-Phe-Gly motif (DFG)-in and αC-in conformation (active state). Type 2 inhibitors inhibit kinases at the ATP site with the DFG-out and αC-in conformation (inactive state). Type 3 inhibitors inhibit kinases at the allosteric site that is located close to the ATP site, and the αC helix usually transits to the 'out' conformation. Type 4 inhibitors inhibit kinases at an allosteric site that is located remote from the ATP site. Type 4 inhibitors also include antibodies and their derivatives that target extracellular domains of receptor tyrosine kinases (RTKs). Type 1.5 inhibitors have been proposed (not shown), and sometimes type 1.5 and type 2 inhibitors are subdivided into types A and B depending on whether the inhibition involves the back pocket, but this classification does not take into account other kinase states. **b** | Additional approaches for kinase targeting. Bivalent inhibitors target kinases simultaneously at two different binding sites (or domains). Covalent inhibitors inhibit kinases by forming a covalent bond with nucleophilic amino acid residues. There are two types of covalent bond formation: irreversible and reversible. Degraders and molecular glues target kinases via recruitment of the ubiquitin (Ub) degradative pathway. E3, E3 ubiquitin ligase; E2, E2 ubiquitin ligase.

function by a degradation mechanism[177]. The kinase CK1α was also identified as a neosubstrate, which contributes to the clinical efficacy of the thalidomide derivative lenalidomide for the treatment of multiple myeloma and 5q-deletion-associated myelodysplastic syndrome[178,179]. Selective degradation can also be induced by conventional kinase inhibitors harbouring surface-exposed moieties. Recently, the CDK inhibitor CR8-a has been shown to recruit the cullin 4 adaptor protein DDB1 (DNA damage-binding protein 1) to the CDK12–cyclin K complex, where it acts as a molecular glue and selective degrader for this kinase[180]. Owing to their low molecular weight, molecular glues can be attractive lead compounds for drug development with very specific modes of action. However, most molecular glues have been discovered serendipitously or have been developed based on natural products, and a rational strategy for their identification is currently lacking.

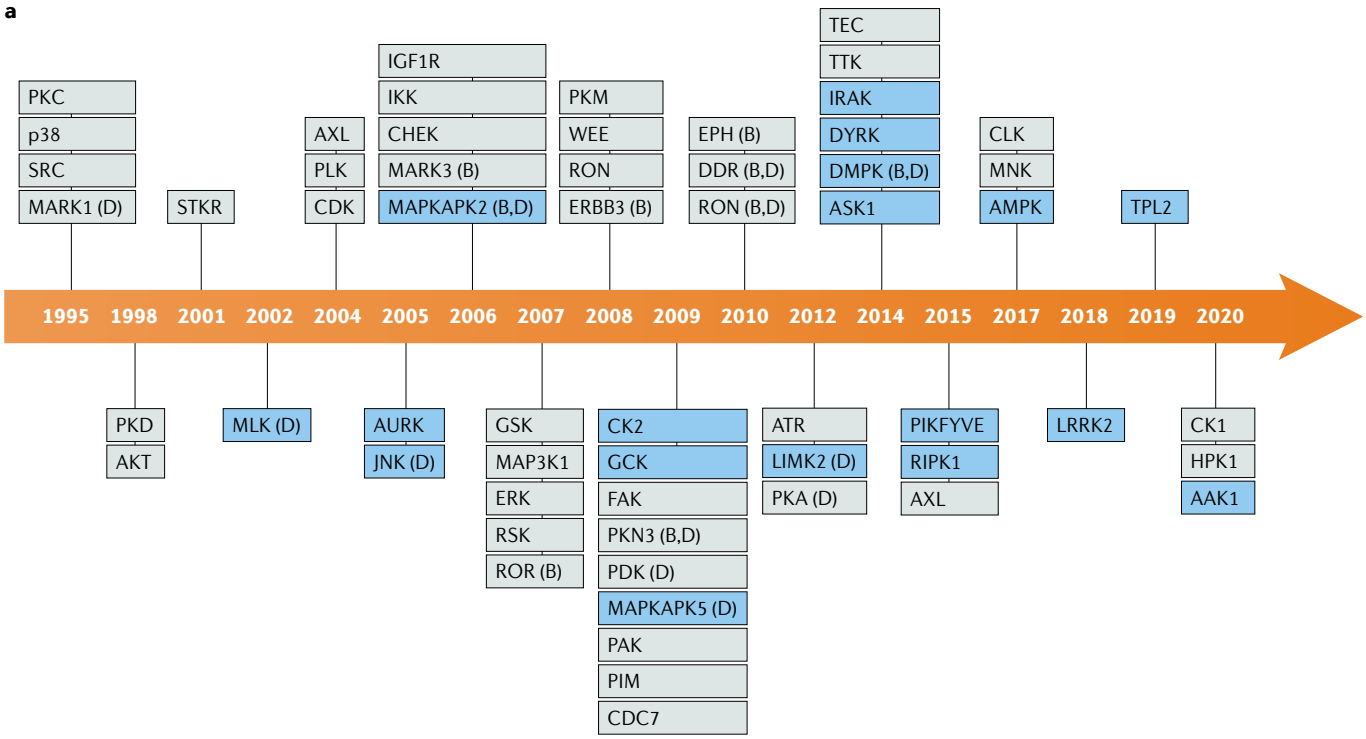
Trends for investigational agents

Our analysis of ClinicalTrials.gov identified nearly 600 kinase-targeting agents registered in clinical trials, which include 475 novel SMKIs (including agents approved by other regulatory agencies and potentially seeking approval by the FDA, but not including the 71 already FDA-approved SMKIs) and also 124 biological agents (see BOX 1 for the dataset details and Supplementary Table 2 for the dataset). In comparison with previous analogous analyses, the number of SMKIs that have reached clinical development has increased more than 200% during the past 5 years, with 177 SMKIs having reached this stage in 2014 (REF.[181]) and 149 in 2010 (REF.[27]).

Of the 475 SMKIs registered in clinical trials, nearly half of these agents — 253 — are currently in development, either in active trials or in the process of beginning new ones, while 222 were determined to not be in active development. Approximately 40% (194) of the kinase inhibitors in clinical trials target 45 novel kinase families that encompass 215 proteins (40% of the kinome), although we identify around 110 of these as the primary targets of kinase inhibitors, so a more accurate estimate is that 20% of the kinome beyond the targets validated by approved drugs is being investigated with agents in clinical development (FIG. 6). This indicates a robust exploration of the novel kinase landscape in comparison with other major target families; for example, the proportion of novel G protein-coupled receptors being investigated with agents in clinical trials was 16% in an analogous recent analysis[182].

Success rates of kinase inhibitors in clinical trials. Using data from 475 investigative agents in more than 3,700 clinical trials and extrapolating trial completion information for the 71 already approved drugs, we estimate that the phase transition probabilities for kinase inhibitors are 72% from phase I to phase II, 66% from phase II to phase III and 86% from phase III to approval and marketing. Phase transition probabilities were calculated using descriptions from Hay et al.[183] that are based on DiMasi and Grabowski[184] studies. Analyses of phase transition success rates are heterogeneous in methods and data, and a direct comparison can be difficult; however, landmark studies that have investigated the success of lead indications for drugs in general have cited rates comparable to ours, such as 67–71% for phase I to II, 40–45% for phase II to III and 64–68% for phase III to New Drug Application (NDA)/Biologic License Application (BLA)[183,185]. Our estimates are much higher than the FDA estimate of clinical research phase success rates for drugs in general, which are approximately 70% for phase I to II, approximately 33% for phase II to III and up to 30% for phase III to the next phase[186]. Interestingly, our results are comparable to estimates made by Walker and Newell[187] more than a decade ago for kinase inhibitors, which calculated 80%, 69% and 85%, respectively, and indicate that kinase inhibitors have continued to have reduced overall attrition rates and continued success in the high-risk transition from phase II to phase III.

The decrease in phase transition probabilities for investigative agents during phase II studies is ubiquitous for both small-molecule and large-molecule therapies across indications, and has been analysed previously[188,189]. Key findings from Morgan et al.[188] indicated lack of clinical efficacy as the principal cause, which can be mitigated by addressing three pharmacological pillars of candidate survival: exposure at the target site, target binding and expression of pharmacological activity. Both the previous analysis of kinase inhibitors[187] and our study indicate less agent attrition in later-stage development than the FDA estimates for drugs in general. This could be due to the repeated targeting of well-understood kinase signalling pathways and the development of more selective inhibitors that effectively inhibit kinase signalling in disease. We also note a similar overall success rate of kinase inhibitors from phase I to approval, which we estimate to be currently around 41%. This is marginally lower than the previous assessment of kinase inhibitors, which estimated an overall success rate of around 47%[187]. However, our study comprises an additional decade of data and about three times as many drugs (546 compared with 137). Additionally, differences in methods of determining agents that are no longer active may affect the analysis (see Supplementary Table 3 for details).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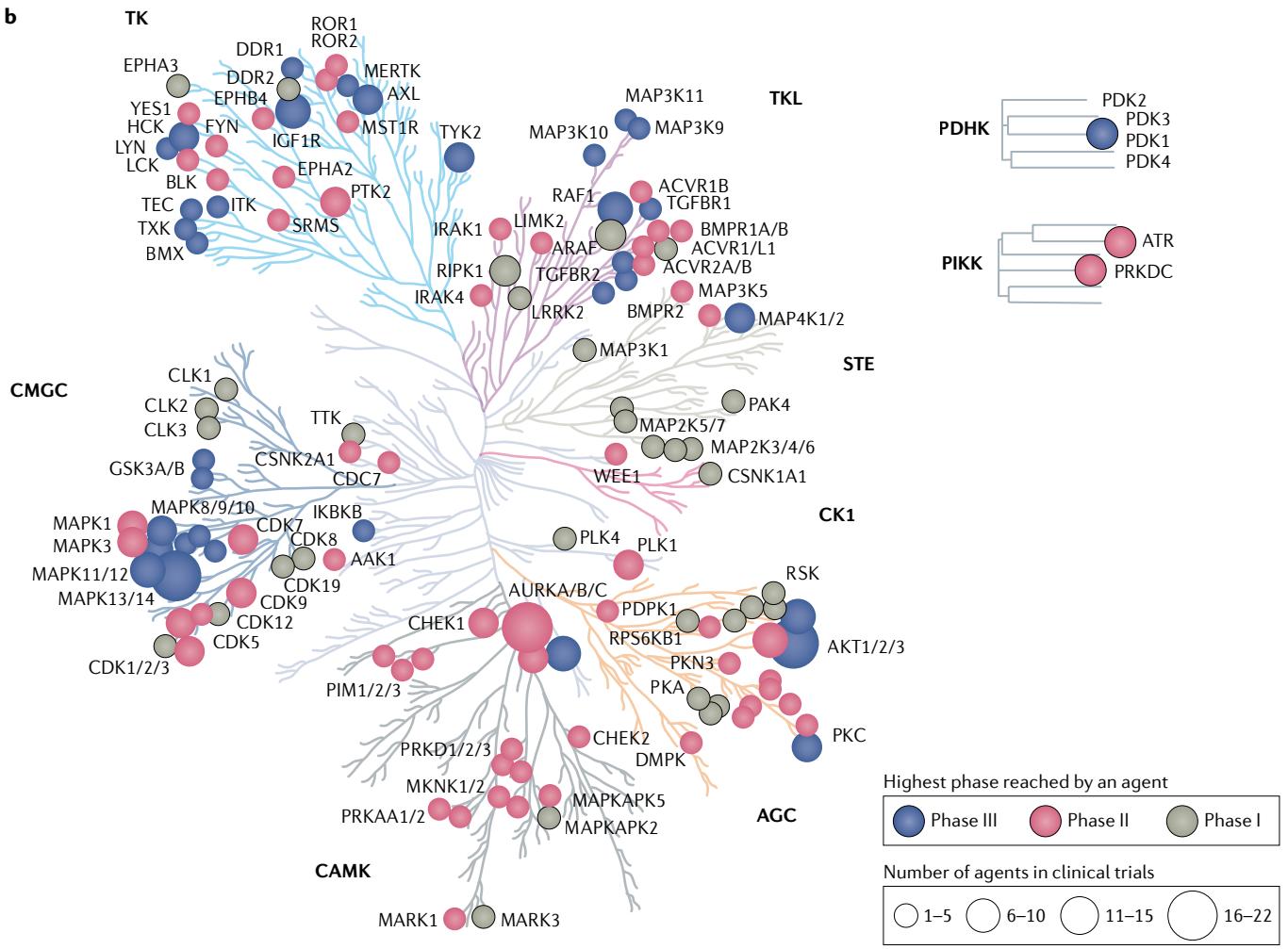
PKN3 (B,D)
PDK (D)
MAPKAPK5 (D)
PAK
PIM
CDC7
ATR
LIMK2 (D)
PKA (D)
PIKFYVE
RIPK1
AXL
LRRK2
CK1
HPK1
AAK1


b
TK
ROR1
ROR2
DDR1
DDR2
EPHA3
EPHB4
MERTK
AXL
YES1
HCK
FYN
IGF1R
MST1R
LYN
LCK
EPHA2
BLK
PTK2
TEC
ITK
TXK
SRMS
BMX
TYK2
TKL
MAP3K11
MAP3K10
MAP3K9
RAF1
ACVR1B
TGFBR1
IRAK1
LIMK2
BMPR1A/B
ARAF
ACVR1/L1
RIPK1
ACVR2A/B
TGFBR2
IRAK4
MAP3K5
LRRK2
BMPR2
MAP4K1/2
MAP3K1
STE
PAK4
MAP2K5/7
MAP2K3/4/6
WEE1
CSNK1A1
CMGC
CLK1
CLK2
CLK3
TTK
GSK3A/B
CSNK2A1
CDC7
MAPK8/9/10
IKBKB
MAPK1
MAPK3
CDK7
CDK8
AAK1
MAPK11/12
CDK19
MAPK13/14
CDK9
CDK12
CDK5
CDK1/2/3
CK1
PLK4
PLK1
RSK
AURKA/B/C
PDPK1
CHEK1
RPS6KB1
AKT1/2/3
PKN3
PIM1/2/3
PKA
PKC
CHEK2
DMPK
PRKD1/2/3
MKNK1/2
MAPKAPK5
PRKAA1/2
MAPKAPK2
AGC
CAMK
MARK1
MARK3
PDHK
PDK2
PDK3
PDK1
PDK4
PIKK
ATR
PRKDC
Highest phase reached by an agent
Phase III
Phase II
Phase I
Number of agents in clinical trials
1–5
6–10
11–15
16–22

◀ Fig. 6 | **Exploring the kinome. a** | Timeline of the year for which agents with novel kinase family targets entered clinical trials. The date is based on the start date of the trial of the earliest investigative agent that targets that kinase family that we could identify. Established targets are not included. For brevity, we present the first instance we identify that an investigative agent has targeted a member of a specific kinase family, although there are several examples where we describe subgroups of these families (for example, the MAPK subgroups JNK and ERK). Kinase targets that are in clinical trials for non-oncology indications are denoted with a blue square. The data include kinase targets of both biologic therapies (denoted with (B)) and small-molecule kinase inhibitors. Kinase targets for which agents are not in active trials and for which no further development has been reported (and are thus presumed discontinued) are marked with a (D). **b** | The kinase targets of the small-molecule kinase inhibitors are mapped onto a phylogenetic tree of the human kinome. Phase status is indicated by the colour of the circle, and the number of agents in clinical trials that target a given kinase is indicated by the size of the circle. CMGC, CDK, MAPK, GSK3 and CLK families; TK, tyrosine kinase.

Kinase families and targets for investigational drugs. Established kinase families and targets continue to be actively investigated in clinical trials with novel agents. Approximately 12% (73 of 599) of the agents in clinical trials target kinases in the HER family to treat various cancers, including 26 novel SMKIs and 47 novel biologics. VEGFRs also continue to be investigated as anticancer targets, with 30 novel SMKIs and nine novel biologics targeting these RTKs in clinical trials. Furthermore, there are 54 PI3K or PI3K–mTOR pathway inhibitors in clinical trials, with six in phase III trials for a range of indications such as lymphomas (buparlisib), activated PI3-kinase delta syndrome (leniolisib) and myelofibrosis (parsaclisib). PI3K and mTOR inhibitors could also have potential for the treatment of rare genetic disorders known as overgrowth syndromes, such as PTEN hamartomas, tuberous sclerosis complex and PIK3CA-related overgrowth spectrum[190–192].

Outside oncology, there are currently 12 SMKIs targeting JAKs in clinical trials for various immunoinflammatory diseases, with seven of them in phase III trials. Of particular interest are the phase III-stage JAK3 inhibitor ritlecitinib (PF-06651600), which received breakthrough therapy designation for alopecia in 2018 after showing promise in a phase II trial (see Related links), and a gut-selective pan-JAK inhibitor (TD-1473) that demonstrated promising results in early clinical trials and is currently in several phase II/III trials[193].

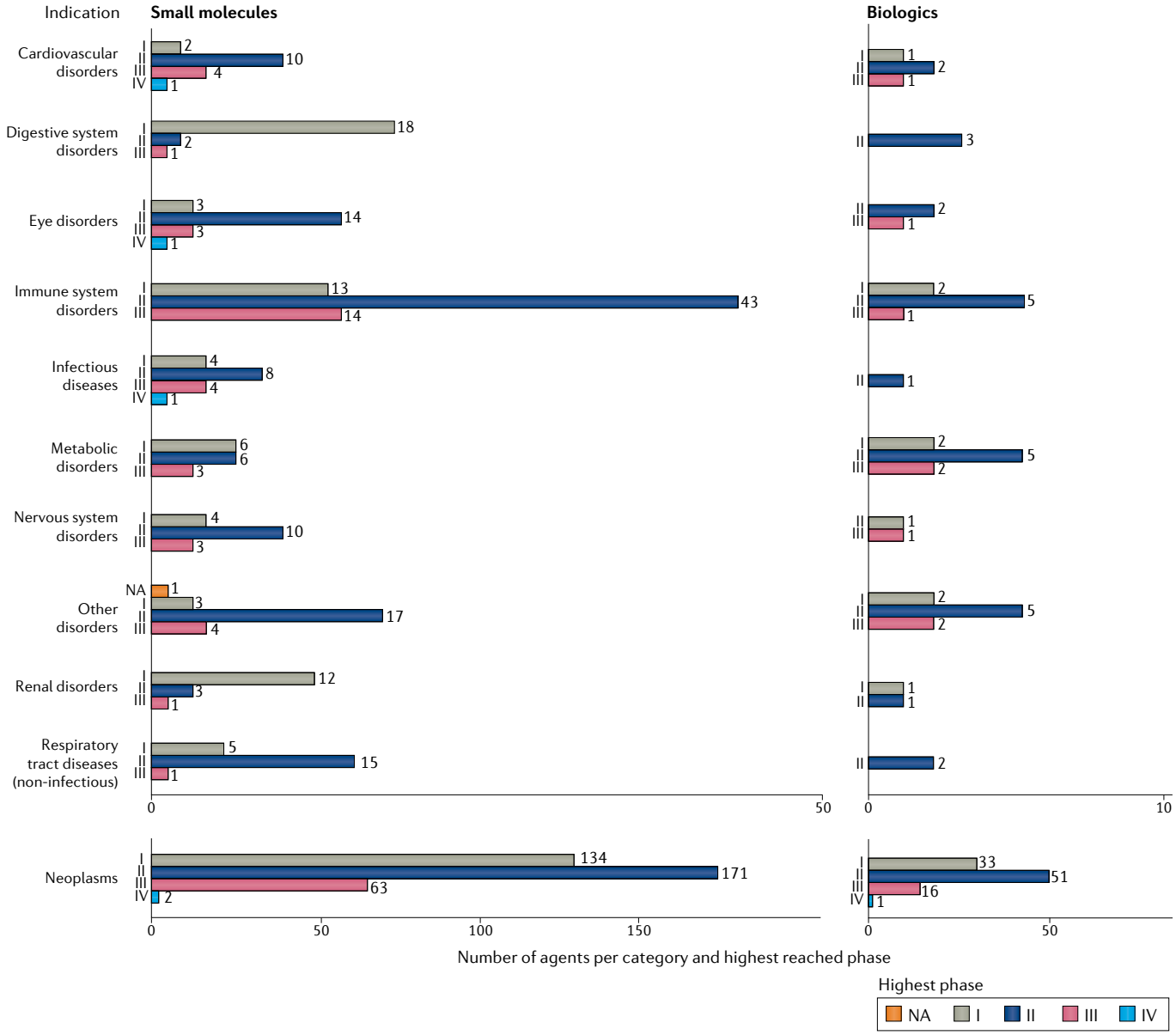
BTK inhibitors have been highly successful in the treatment of B cell-related blood cancers (see above), and the role of B cells in autoimmune diseases has also led to BTK inhibitors being investigated for such diseases. Seven BTK inhibitors are being studied in phase II trials for systemic lupus erythematosus, and two BTK inhibitors (evobrutinib and SAR442168) are in phase II and phase III trials for multiple sclerosis, with evobrutinib showing marginal effectiveness with limited toxicities in a previous trial[194,195].

As noted above, at least 45 different novel kinase families are being targeted in clinical trials, which include more than 110 different kinases identified as the primary targets. Several families have been targeted by a particularly high number of agents in clinical development, with inhibitors undergoing evolutions of chemical types and targeted indications. Interestingly, novel families in the TK group are less targeted in comparison with the other major kinase groups. In the CMGC (CDK, MAPK, GSK3 and CLK families) group, nearly 50 agents have entered trials targeting members that have been studied in clinical trials for a long time, such as the mitogen-activated protein kinase (MAPK) p38 family, the ERK family and members of the cyclin-dependent kinase (CDK) family. More than 20 agents have targeted the Aurora kinase family, while in the AGC group, 17 inhibitors have targeted the AKT (protein kinase B, PKB) family. Further details of all targeted families are provided in Supplementary Fig. 1. Selected examples of the novel families are discussed below, subdivided into those that are being investigated for oncology indications and those that are being investigated for indications beyond oncology. FIGURE 7 summarizes the disease indications of kinase-targeted agents in clinical trials.

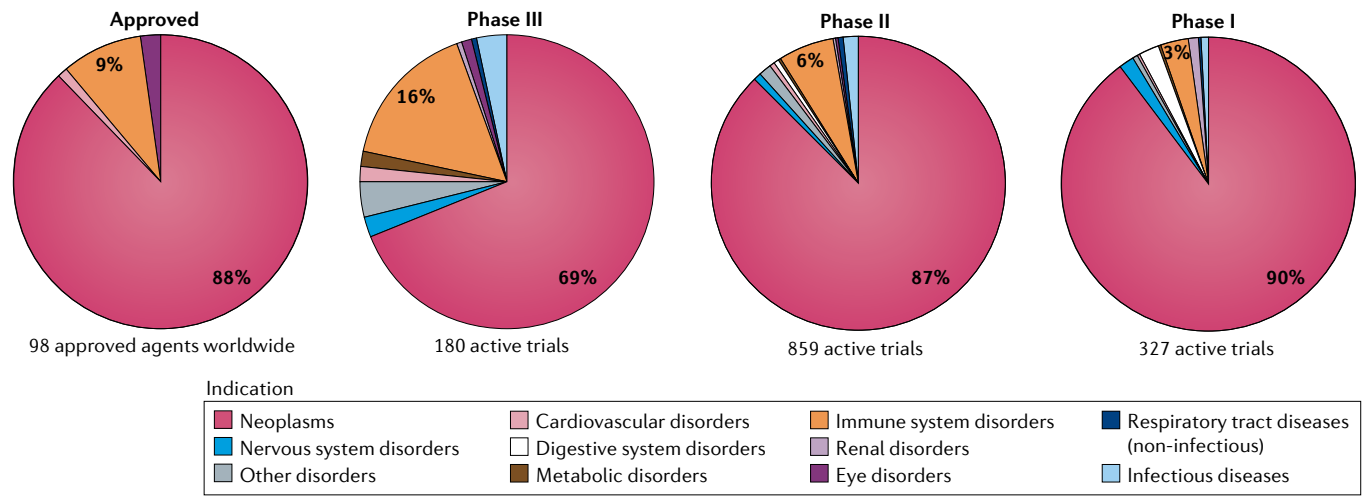
Investigational kinase inhibitors with novel targets in oncology. The Aurora kinase family (Aurora kinase A, Aurora kinase B and Aurora kinase C) has been the most targeted kinase family for which an agent has not yet been approved, with 22 agents having entered clinical trials. The role of these kinases in cell division and their overexpression in a broad range of human tumours has long stimulated interest in their potential as therapeutic targets[196], but the development of more than 80% (18) of Aurora kinase inhibitors that have reached clinical trials has been discontinued. First-generation Aurora kinase inhibitors were tested in solid tumours, but results from trials showed limited efficacy and high toxicity, potentially because cell proliferation in solid tumours is relatively slow and drug exposure through a number of cell cycles is needed to induce maximum tumour antiproliferation effects[197]. These clinical disappointments brought a change in strategy, and second-generation, isoform-selective Aurora kinase inhibitors are being investigated for haematological malignancies based on the rationale that they have higher homogeneity and higher proliferation rates relative to solid tumours[197]. Currently, four Aurora kinase inhibitors are in trials as monotherapies or in combinations in leukaemias such as acute myeloid leukaemia and chronic lymphocytic leukaemia, and also in solid tumours.

The CDK family includes 20 members, which can be functionally classified into families that act in controlling cell cycle transitions (including CDK1, CDK2, CDK4, CDK5 and CDK6) and regulating transcription (including CDK8, CDK9, CDK11 and CDK20), although both of these activities are present in several family members such as CDK7 (REF.[66]). On the basis of their roles in the control of cell division and that cancers exhibit dysregulation of the cell cycle, CDKs have been therapeutic targets for 20 years. The first-generation CDK inhibitors, such as the extensively studied flavopiridol (alvocidib), which targets most CDKs, showed insufficient efficacy and significant toxicity[198]. Development of next-generation inhibitors led to the successful validation of selectively targeting CDK4 and CDK6 for the treatment of breast cancer in 2015 with the FDA approval of palbociclib, followed by abemaciclib and ribociclib in 2017 (see above). Members of the CDK family continue to be intensively investigated in clinical trials, with five novel SMKIs that target the CDK4

a **Molecule type**



b **Ongoing trials and approved agents**



◀ Fig. 7 | **Disease indications of kinase-targeting agents in clinical trials. a** | Kinase inhibitors in clinical trials. This graph illustrates the number of kinase inhibitors per disease category in clinical trials. The length of the bar is proportional to the number of agents. The colour of the bars and roman numerals correspond to the highest achieved phase by each agent for a particular category of indications. Some agents may belong to several categories simultaneously and have a different highest phase reached for each category. The neoplasms category has a separate scale. Biologics and small molecules are divided into subfigures. For this chart, both active and inactive clinical trials have been included. **b** | Indications in active clinical trials. The pie charts show the distribution of indications in ongoing clinical trials and among approved agents. The colours of the slices correspond to the category of indication.

family in clinical trials, and an additional 12 SMKIs targeting CDK7, CDK8/CDK19, and CDK2 and CDK9 either selectively or in combinations with other agents currently in phase I and II trials.

Tumour cells are known to develop resistance to many cancer therapies via the DNA damage response (DDR), which supports DNA repair[199]. Several SMKIs that have been specifically developed to combat this resistance mechanism by targeting kinases involved in the DDR are being investigated in clinical trials, including ataxia–telangiectasia and Rad3 related (ATR), DNA-dependent protein kinase catalytic subunit (DNA-PK) and checkpoint kinase 1 (CHK1)[200]. Currently, four SMKIs that target ATR, DNA-PK or CHK1 are in 20 phase II trials for different types of malignancy.

Investigational kinase inhibitors with novel targets beyond oncology. Currently, more than 40 kinase inhibitors are in clinical trials for immune system disorders such as rheumatoid arthritis, psoriasis, systemic lupus erythematosus, inflammatory bowel disease, multiple sclerosis and alopecia areata, with 12 agents in phase III trials. Some of these agents inhibit established targets such as JAKs, as noted above, but there are also novel kinase targets being pursued for inflammatory diseases, including IL-1 receptor-associated kinase 4 (IRAK4), receptor-interacting serine/threonine protein kinase 1 (RIPK1), tumour progression locus 2 (TPL2) and tyrosine kinase 2 (TYK2).

There are currently 16 SMKIs in clinical trials for rheumatoid arthritis. All three of the agents in phase III trials have established targets, but one SMKI in a phase II trial has a novel mode of action: PF-06650833 targets IRAK4, thereby inhibiting signalling by IL-1, which is a potent pro-inflammatory cytokine that is involved in the pathogenesis of various autoimmune diseases[201].

RIPK1 is important in mediating inflammation by another potent pro-inflammatory cytokine — tumour necrosis factor (TNF) — and increased RIPK1 activity is associated with neuroinflammation and cell necroptosis[202]. The first agent targeting RIPK1 entered clinical trials in 2015, and there are now three RIPK1 SMKIs in clinical trials. The furthest advanced of these is GSK2982772 (REF.[203]), which has completed eight phase I and II clinical trials for the treatment of various autoimmune diseases and is in a phase I trial for moderate to severe psoriasis.

Accumulating evidence has described the multifaceted ways in which TPL2 is involved in inflammation, including positive regulation of MEK1/MEK2 to activate ERK1/ERK2 signalling[204] and positive regulation of p38α and p38δ in neutrophils[205]. Hence, it is speculated that inhibition of its activity may be beneficial in treating inflammatory diseases such as rheumatoid arthritis and psoriasis[205] as well as in multiple sclerosis[206]. One TPL2 inhibitor, GS-4875, has entered clinical trials and is currently in a phase II study for the treatment of ulcerative colitis.

Overall, 15 agents are in clinical trials for the treatment of psoriasis through inhibition of several kinases in addition to RIPK1, including JAK1, TYK2, IRAK4 and ROCK2. Interestingly, JAK kinases (JAK1–3 and TYK2) harbour two kinase domains: a catalytically active JH1 domain and the pseudokinase domain JH2, which has an important role in TYK2 activation. All clinically approved JAK inhibitors are JH1 inhibitors that show moderate selectivity within the JH1 domains, a property that has been linked to serious side effects. The pseudokinase domain of TYK2, however, shows some structural features that have been explored for inhibitor development. The leading agents in this area are the two TYK2 JH2 inhibitors deucravacitinib (BMS-986165) and BMS-986202 (REF.[207]). These have both been shown to potently prevent TYK2 activation, with remarkable selectivity over other JAK family members and the kinome, and are currently in phase III trials. Side effects have been reported for deucravacitinib, including haemorrhage and anaemia[208], but if these agents prove sufficiently safe and maintain the same effectiveness in current phase III trials, they are likely to be approved in the near future, thereby becoming the first inhibitors that target an inactive pseudokinase domain.

CDC-like kinase 2 (CLK2) and dual-specificity tyrosine phosphorylation-regulated kinase 1A (DYRK1A) are regulators of Wnt signalling, chondrogenesis and inflammation, and inhibiting these kinases has demonstrated potential disease-modifying effects for osteoarthritis[209]. The small-molecule dual kinase inhibitor lorecivivint has shown promising osteoarthritis therapeutic effects by inducing chondrocyte differentiation, cartilage regeneration and protection, and improving joint health score[210]. It is currently in three phase III trials for treatment of knee osteoarthritis, and results from a phase II trial demonstrate the disease-modifying abilities of lorecivivint and its potential as a first-in-class drug[211].

The p38 MAP kinases were originally discovered as key regulators of inflammatory cytokine biosynthesis[212] in the 1990s, and they have since been extensively pursued for inflammatory diseases such as rheumatoid arthritis, without any inhibitors directed at these kinases being approved so far. At least 20 such agents have entered clinical trials, but the development of 16 (80%) of these agents seems to have been discontinued. First-generation SMKIs targeted p38α and p38β[213], but off-target effects, potency issues, compensatory pathways and toxicity have limited their clinical development[214]. Second-generation inhibitors were developed, such as the slow off-rate inhibitor BIRB 796 (REF.[215]), which have also not been successful[216]. However, a recent review notes a shift in p38 research to focus on more acute inflammatory disorders including chronic obstructive pulmonary disease or asthma[217]. Furthermore, p38 kinase function has been

linked to several neurological and psychiatric disorders, particularly when associated with neuroinflammatory responses[218]. One of the most recent p38 SMKIs to enter clinical trials, neflamapimod (VX-745), is in a novel class of p38 inhibitors that contain a bicyclic heterocycle core with increased selectivity for p38α over p38β[219]. Neflamapimod demonstrated statistically significant cognitive improvements in patients with Alzheimer disease in phase II trials (see Related links), and is currently in three phase II trials for the treatment of Huntington disease (NCT03980938), Alzheimer disease (NCT03435861) and dementia with Lewy bodies (NCT04001517).

Exploration of kinase inhibitors in nervous system disorders is at an early stage, with no approved inhibitors and the majority of drugs still in phase I or II trials. Several agents in addition to neflamapimod are in clinical trials that target various aspects of pro-inflammatory cytokine production and immune response, based on the evidence for the importance of inflammation in disorders such as Alzheimer disease[220]. DNL747 inhibits RIPK1, edicotinib (JNJ-40346527) targets colony-stimulating factor 1 receptor (CSF1R), and masitinib binds to KIT and other RTKs. In December 2020, masitinib was reported to provide clinical benefit as an add-on therapy to cholinesterase inhibitors compared with placebo in a phase II/III trial (see Related links), and a confirmatory trial should be initiated soon. Another interesting agent is saracatinib (AZD0530), which inhibits Fyn kinase, a member of the Src family. This may be a promising strategy for Alzheimer disease therapy because its downstream pathway is believed to underlie the toxicity of amyloid-β, a hallmark of the disease[221]. Interestingly, saracatinib has also been analysed as a potential therapeutic candidate for alcohol use disorder in a phase II trial (NCT02955186) because Fyn signalling is implicated in alcohol drinking behaviour[222].

Another interesting kinase target for nervous system disorders is leucine-rich repeat kinase 2 (LRRK2), as activating mutations in *LRRK2* are the most common autosomal-dominant cause of inherited Parkinson disease[223,224]. Three LRRK2 inhibitors have now entered phase I trials for the treatment of Parkinson disease: two SMKIs (DNL201 and DNL151) and one antisense oligonucleotide (ION-859). So far, results for DNL201 show that it was well tolerated (see Related links), and DNL151 is scheduled to advance in clinical trials. A trial of ION-859 is currently recruiting patients.

Adaptor-associated kinase 1 (AAK1) is receiving considerable attention as a target for the treatment of neuropathic pain that might provide the basis for a new class of analgesics[225]. Studies in animal models showed that inhibition of AAK1 was mechanistically linked to α2 adrenergic signalling rather than opioid receptors to reduce pain responses[225]. One novel AAK1 inhibitor has entered trials; the small molecule LX9211 is currently in two phase II trials for neuralgia and diabetic neuropathy and recently received fast-track designation from the FDA (see Related links).

Kinases for which the development of inhibitors has been discontinued. There are at least 11 kinase families and approximately 20 kinase targets for which the development of inhibitors appears to have stalled or been abandoned, as there are no agents in active trials, and many of the latest status reports from these agents are from more than 5 years ago. The discontinued agents and kinase targets in our dataset are provided in Supplementary Table 4, and we discuss selected examples briefly here.

One important family is the Jun N-terminal kinase (JNK) family, which are MAP kinases involved in regulating cell survival and proliferation in response to cytokines and growth factors that have been implicated in a range of diseases including cancer, immune diseases and neurodegenerative disorders[226]. Four JNK inhibitors reached clinical trials for treatment of myeloid leukaemia in 2005 (CC-401; phase I), fibrotic disorders in 2011 (CC-930; phase II), endometriosis in 2012 (PGL5001; phase II) and hearing loss in 2008 (AM-111; phase III trial completed in 2017) but have not progressed further. Common limitations of kinase inhibitors including toxicity and lack of kinase specificity have been cited as probable reasons[227].

A more recent example of a novel kinase target in clinical trials for which drug development appears to be suspended is the monopolar spindle 1 (MPS1) kinase, which has an important role in cell division[228]. Two ATP-competitive kinase inhibitors, BAY1161909 and BAY1217389, showed modest efficacy as single agents in preclinical models but synergistic effects in combination with paclitaxel[229] and entered phase I trials in combination with paclitaxel. While the development of BAY1161909 was terminated as a strategic decision, BAY1217389 completed the trial and demonstrated good tolerability with manageable adverse effects and preliminary evidence of efficacy[230], but its development appears to have been recently discontinued.

Outlook

Although there have been substantial advances in kinase drug discovery, there are still many challenges and opportunities in this field. In the oncology area, where kinase inhibitors have become one of the cornerstones of cancer therapy in the two decades since the approval of imatinib, combating drug resistance is imperative for long-term effectiveness, and several such strategies are being developed. The design of drugs that target common and rare kinase driver mutations is one approach being used to combat drug resistance, as illustrated by the development of multiple generations of kinase inhibitors for NSCLC discussed above. The use of combination regimens is another approach, as illustrated by the application of combinations of BRAF and MEK inhibitors in the treatment of melanoma. Furthermore, as multiple kinase inhibitors are being combined to create effective, durable, therapies, rational development of single molecules that inhibit multiple relevant kinases may be another ambitious approach.

There are also several functional challenges such as the characterization of genetic aberrations in the cancer kinome, as well as identification of bona fide drivers responsible for tumour development. A careful analysis of biological pathways for kinases and their relation to cell biology and therapeutic intervention is of high

Box 3 | **Probing the kinome with tool compounds**

Developing tool compounds to assess the bioactivity and selectivity of kinase inhibitors is particularly important in kinase research owing to the propensity of kinase inhibitors to cross-react with multiple kinases. Tool compounds have been successfully used to create kinase inhibitors for clinical development; for example, a highly potent and monoselective inhibitor for RIPK1 was recently reported (GSK′481)[254], which was then optimized into a first-in-class clinical candidate for treatment of inflammatory diseases (GSK2982772) that is currently in clinical trials[203].

We queried ChemicalProbes.org to investigate tool compounds that are publicly available for kinase drug discovery. Results included 85 kinase inhibitors classified as historical compounds; that is, small molecules that are typically non-selective or not sufficiently potent compared with other chemical probes. This means that 35% of their historical compound dataset are kinase inhibitors, which indicates not only the incredible amount of work that has been dedicated to kinase inhibitor development, but also the inherent difficulty in creating selective and potent kinase inhibitors.

Results also included 127 chemical probes, with ten of these compounds currently undergoing curation (Supplementary Table 5). At least 22 probes target unexplored kinases that have not yet reached clinical development as a primary target and at least 56 probes target kinases that are identified as a primary target of a kinase inhibitor in clinical development. This means that more than 75 tool compounds are available to optimize and advance kinase inhibitors in clinical development. Additionally, 49 probes that target already validated kinases were classified.

Aside from tyrosine kinases, tool compounds that target the CDK, MAPK, GSK and CLK (CMGC) families and the 'other kinases' group are the most common. Nearly 40 probes have recent expert review comments posted in 2020, including several that target kinases that have not yet reached clinical trials such as the serine/threonine protein kinases TBK1 and NEK2, mitotic checkpoint serine/threonine protein kinase BUB1 and activated CDC42 kinase 1 (ACK1). These kinases have been identified in diseases that are amenable to kinase inhibition, including neoplasms, skin disorders and neurological disorders such as dementia and amyotrophic lateral sclerosis.

importance, as a kinase might be a tumour suppressor in non-malignant cells yet mediate tumour survival in malignant cells. For example, it has been known for a long time that the ATR–ATM pathway initiates apoptosis following DNA damage, and its impairment leads to tumorigenesis[231]. However, it was discovered later that this pathway is also exploited by tumour cells to resist the effects of DNA-damaging cancer therapies, and this has led to the development of inhibitors of kinases such as ATR, DNA-PK and CHK1, which are currently in clinical trials (see above).

Our analysis indicates that at least 70% (approximately 400 kinases) of the kinome is still unexplored in drug development in the sense that no agents for which these kinases are considered primary targets have entered clinical trials. Several metrics can provide some indication of the relative levels of exploration of members of the kinase family, including the literature output, the number of compounds that inhibit each kinase in the database Chembl and the number of available resolved structures in the Protein Data Bank. An ongoing initiative integrating such knowledge is the NIH Illuminating the Druggable Genome (IDG) project, which collates evidence from multiple domains to map the knowledge gaps around potential drug targets and also prompt investigation into understudied but potentially druggable proteins. IDG presents the information in the user interface Pharos[232] and also highlights interesting understudied targets (see Target Watch in Related links). IDG has classified 635 protein kinases according to different target development levels, and they estimate that 3% (21 kinases; T_{dark}) of the kinome is characterized as 'dark', where virtually nothing is known about these kinases, and about 30% (188 kinases; T_{bio}) do not have known drug or small-molecule activities that pass their specific activity thresholds, which include scores from text-mining the literature and Gene Reference Into Function annotations. Interestingly, understudied proteins including those identified in T_{dark} and T_{bio} classes may be frequently altered in different types of cancer, indicating their potential functional importance for previously uncharacterized tumour cell phenotypes[233]. Furthermore, in a recent clinical study that assessed transcriptional alterations in triple-negative breast cancer tumours, both T_{dark} and T_{bio} understudied proteins were found to be transcriptionally altered, demonstrating that they are co-regulated transcriptionally with other kinases that have been more investigated (for example, kinases targeted by approved drugs)[233,234]. This illustrates the potential therapeutic importance of identifying and further investigating these understudied kinases.

Overall, the current state of research on kinases indicates strong publicly available knowledge, with wide structural coverage and also a substantial number of chemical probes available to investigate the kinome (BOX 3). These efforts to elucidate kinase structures, probes and target characterizations, as well as many other aspects of drug discovery, have been facilitated to a large extent by the Structural Genomics Consortium, which is an open collaborative network of scientists and pharmaceutical companies with the focus to provide research output available to the scientific community. To continue to expand kinase drug discovery, there is a need for further development and refinement of efficient compound screening and profiling technologies. In particular, methods that can identify new chemical matter, including natural compounds, need to be addressed. Additionally, there are challenges in obtaining target selectivity to reduce off-target toxicity. The development of inhibitors with different types of binding mode, such as allosteric and covalent inhibitors, and targeted degraders such as PROTACs and molecular glues (FIG. 5), could have a key role in the next two decades of kinase drug discovery.

Data availability

Data are provided in the Supplementary information. Supplementary Table 1 provides information on protein kinase inhibitors approved by selected regulatory agencies (FDA, EMA, NMPA, PMDA and MFDS) and their primary therapeutic targets. Supplementary Table 2 provides information on agents in clinical trials that target protein kinase signalling pathways. Supplementary Table 3 provides success rates of FDA-approved small-molecule kinase inhibitors in clinical trials. Supplementary Table 4 provides information on kinase targets for which modulators in clinical trials are presumed discontinued. Supplementary Table 5 provides information on chemical probes and historical compounds for kinases. Supplementary Fig. 1 provides information on active and not active small-molecule kinase inhibitors targeting established and novel kinases.

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