

INTERNATIONAL UNION OF BASIC AND CLINICAL PHARMACOLOGY REVIEW

Ten things you should know about protein kinases: IUPHAR Review 14

Correspondence

Doriano Fabbro, PIQUR
Therapeutics, Hochbergerstrasse
60C, CH-4057 Basel, Switzerland.
E-mail:
doriano.fabbro@piqur.com

Received

5 October 2014

Revised

31 December 2014

Accepted

20 January 2015

Doriano Fabbro¹, Sandra W Cowan-Jacob² and Henrik Moebitz²

¹PIQUR Therapeutics, Basel, Switzerland, and ²Novartis Institutes of Biomedical Research, Basel, Switzerland

Many human malignancies are associated with aberrant regulation of protein or lipid kinases due to mutations, chromosomal rearrangements and/or gene amplification. Protein and lipid kinases represent an important target class for treating human disorders. This review focus on 'the 10 things you should know about protein kinases and their inhibitors', including a short introduction on the history of protein kinases and their inhibitors and ending with a perspective on kinase drug discovery. Although the '10 things' have been, to a certain extent, chosen arbitrarily, they cover in a comprehensive way the past and present efforts in kinase drug discovery and summarize the *status quo* of the current kinase inhibitors as well as knowledge about kinase structure and binding modes. Besides describing the potentials of protein kinase inhibitors as drugs, this review also focus on their limitations, particularly on how to circumvent emerging resistance against kinase inhibitors in oncological indications.

Abbreviations

ABL, Abelson kinase; Akt, protein kinase B or kinase from the transforming oncogene Akt8; ALK, anaplastic lymphoma kinase; A-loop, activation loop also called activation segment; BTK, Bruton tyrosine kinase; Catalytic loop, Y/HRD or Tyr/His-Arg-Asp; CHEK1 (CHK1), checkpoint kinase-1; DGF-motif, Aspartate-Glycine-Phenylalanine or Asp-Gly-Phe; EGFR, epidermal growth factor receptor; ELK, eukaryotic-like kinase; ePK, eukaryotic protein kinases; FAK, focal adhesion kinase; FDA, Food and Drug Administration; Fes, Feline sarcoma oncogene kinase; FGFR1, fibroblast growth factor receptor-1; FLT3, fetal liver kinase-3; GIST, gastrointestinal stromal tumours; G-loop, glycine-rich loop also known as P-loop Gly-loop; GSK3 β , glycogen synthase kinase-3 β ; HES, hyper-eosinophilic syndrome; JH2, Jak homology domain-2; LKB1, serine/threonine-protein kinase STK11; MAP2K, mitogen activated kinase kinase; MAP3K, mitogen activated kinase kinase kinase; MAP4K, mitogen activated kinase kinase kinase kinase; MAPK, mitogen activated kinase; MEK1, mitogen activated kinase kinase-1; MET, mesenchymal epithelial transition factor or hepatocyte growth or scatter factor receptor; mTOR, mammalian target of rapamycin; Myr-pocket, myristate-pocket; P450, cytochromes P450 (CYPs); PDGFR, platelet-derived growth factor receptor; PDK1, 3-phosphoinositide-dependent protein kinase-1; PI, phosphatidyl-inositol; PIF, PDK1 interacting fragment; PK/PD, pharmacokinetic/pharmacodynamic; PKL, protein kinase-like; pSer, pThr, pTyr, phospho-Serine (pS), phospho-Threonine (pT), phospho-Tyrosine (pY); P-site, peptide-site; PTEN, phosphatase and tensin homologue; RAF, rapidly accelerated fibrosarcoma; SMAD, SMAD is the composite of MAD form drosophila Mothers Against Decapentaplegic and SMA of *Caenorhabditis elegans* (from gene *sma* for small body size); STPK, serine- and threonine-specific protein kinase; STRAD1, STE20-related adapter alpha; TPK, tyrosine protein kinase; TrkB or NTRK-2, tropomyosin receptor kinase B or neurotrophin receptor kinase-2

Tables of Links

TARGETS		
Catalytic receptors ^a	Enzymes ^b	
ALK	ABL (Abl)	MAPK
AXL	Akt (PKB)	MEK1
CSF1R	AMPK	MLKL
EGFR	Aurora kinase	mTOR
FGFR1	B-Raf (BRAF)	PDK1
FLT3	BTK	PHK
HER2 (Neu)	CHEK1 (CHK1)	PI3Kδ
IGF1R	ELK (EphB1)	PIK3CA
Insulin receptor	FAK	PKCζ
KIT	Fes	PTEN
MET (c-Met)	Glucokinase	PTK
PDGFRα	GSK3β	RAF
PDGFRβ	Haspin	Ribosomal S6 kinase
RET	Hck	ROCK
ROS1	JAK2	STK11
TIE2	JNK1	STRAD1
TrkB	LKB1	Src

LIGANDS	
ADP	Lapatinib
ATP	Myristate
Afatinib	Nilotinib
AZD6244	Nintedanib
Crizotinib	Pertuzumab
Cyclosporine	Ponatinib
Dabrafenib	Sirolimus (rapamycin)
Dasatinib	Sorafenib
Erlotinib	Staurosporine
Fasudil (HA1077)	Sunitinib
Gefitinib	Tofacitinib
GNF-2	Trametinib
Ibrutinib	Trastuzumab
Imatinib	Vemurafenib

These Tables list key protein targets and ligands in this article which are hyperlinked to corresponding entries in <http://www.guidetopharmacology.org>, the common portal for data from the IUPHAR/BPS Guide to PHARMACOLOGY (Pawson *et al.*, 2014) and are permanently archived in the Concise Guide to PHARMACOLOGY 2013/14 (^{a,b}Alexander *et al.*, 2013a,b).

Short history on protein kinases and inhibitors

Post-translational modifications such as phosphorylation, glycosylation, ubiquitination, nitrosylation, acylation, methylation, lipidation and proteolysis, which are known to increase the diversity of the proteome, influence various aspects of normal and pathological physiology (Walsh *et al.*, 2005; Liu *et al.*, 2013). Kinases carry out the phosphorylation reactions by transferring the gamma phosphate of ATP onto hydroxyl groups of various substrates including lipids, sugars or amino acids and is reversed by the corresponding phosphatases. Phosphorylation plays a pivotal role in many cellular and extracellular processes (Blume-Jensen and Hunter, 2001; Cohen, 2001; Walsh *et al.*, 2005; Kannan *et al.*, 2007; Ubersax and Ferrell, 2007). While bacterial signalling occurs through His-Asp kinases and some eukaryotic-like proteins as well as small molecule kinases [eukaryotic protein kinase (ePK)-like kinases or eukaryotic-like kinase (ELK)], the protein kinases of eukaryotes which include the ePKs phosphorylate either tyrosine (TPKs; tyrosine-specific protein kinases), serine/threonine (STPKs; Ser-/Thr-specific protein kinases) or both tyrosine and threonine (dual-specificity protein kinases) (Cohen, 2001; 2002b; Knelly, 2002; 2003; Kannan *et al.*, 2007; Ubersax and Ferrell, 2007). In addition, eukaryotes have kinases that specifically phosphorylate small molecules, including lipids and sugars (Figure 1A) (Kannan *et al.*, 2007; Yuan and Cantley, 2008; Bornancin, 2011; Kunkel *et al.*,

2013). Aberrant phosphorylation in eukaryotes is associated with a variety of disorders ranging from cancer to inflammatory diseases, diabetes, infectious diseases, cardiovascular disorders, cell growth and survival (Blume-Jensen and Hunter, 2001; Cohen, 2001; Walsh *et al.*, 2005; Ubersax and Ferrell, 2007; Lahiry *et al.*, 2010).

The first phosphorylation of proteins was described for casein [by phosphorylase kinase (PHK)] in 1954 (reviewed in Cohen, 2002a). In the late 1970s, only a handful of biochemically characterized STPKs were known against which some inhibitors were identified that were neither potent nor selective (Figure 1A) (Glossmann *et al.*, 1981; Hidaka *et al.*, 1984; Inagaki *et al.*, 1986; Davies *et al.*, 2000). The identification of PKCs as receptors for tumour-promoting phorbol esters, together with the discovery of TPKs as oncogenes in the 1980s, with the advent of molecular cloning led to the initiation of more rational kinase drug discovery approaches. The foundation for the medicinal chemistry on kinase inhibitors at that time was derived from just a few lead compounds, including the natural compound staurosporine and the synthetic tyrphostins (Tamaoki *et al.*, 1986; Levitzki, 1990) (Figure 1B). The first protein kinase inhibitor was fasudil (HA-1077), which was approved in Japan in 1995 for cerebral vasospasm (Shibuya and Suzuki, 1993). Fasudil was followed by sirolimus (Rapamune), the first allosteric kinase inhibitor, which was approved in 1999 for use in combination with cyclosporine for the prevention of organ rejection in patients receiving renal transplants (Kelly *et al.*, 1997; Vasquez, 2000). The target of this natural compound, the kinase mTOR

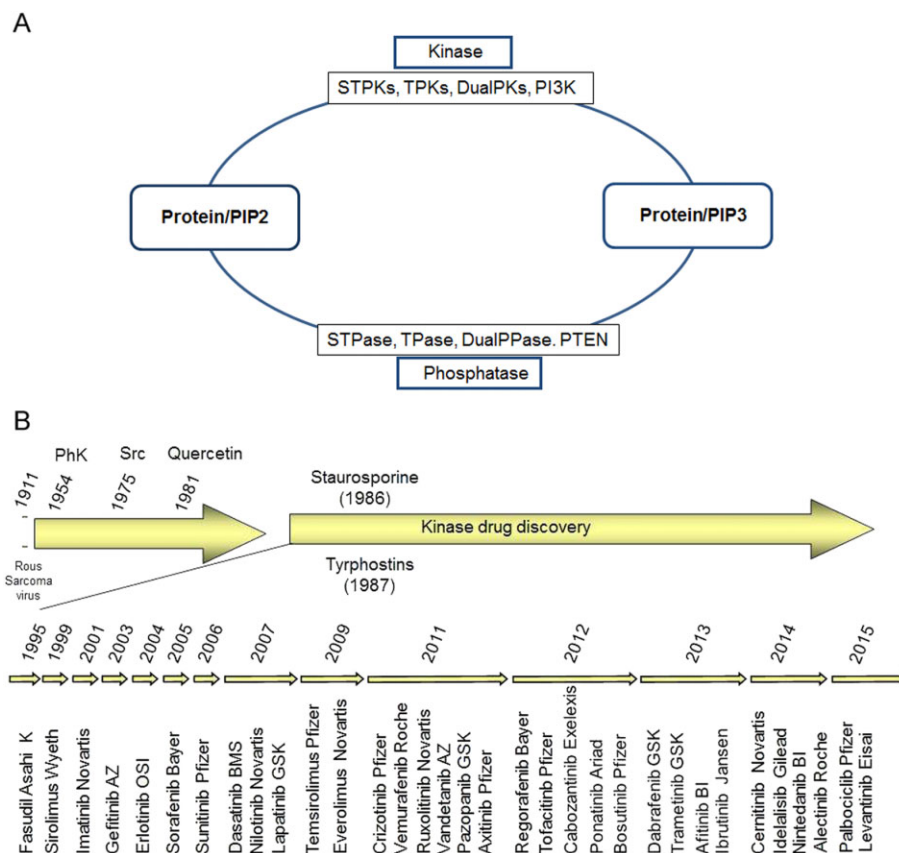


Figure 1

(A) Reversible phosphorylation and (B) kinase drug discovery. For explanation, see text. DualPase, dual-specificity phosphatases; DualPK, dual-specificity kinases; PI, phosphatidylinositol; PIP, phosphatidylinositolphosphate; PI3K, phosphatidylinositol kinases; STPase, Ser/Thr-specific phosphatases; STPK, Ser/Thr-specific kinases; TPK, Tyr-specific kinases.

(mammalian target of rapamycin), was discovered by a genetic screen just a few years before (Kunz *et al.*, 1993).

The first economically successful protein kinase inhibitor imatinib (CGP57148, STI571, Glivec, Gleevec) designed to inhibit the Abelson (ABL) kinase in the context of the BCR-ABL translocation was approved in 2001 for chronic myeloid leukaemia (CML) (Figure 1B) (Buchdunger *et al.*, 2001). The success of imatinib is due to its efficacy during the chronic phase of CML, which is an almost monogenic BCR-ABL-driven myeloproliferative disorder. Imatinib is much less effective against the more aggressive disease state of CML, the blast crisis, an acute leukaemia, which marks the fatal end stage of the disease (Goldman and Druker, 2001; Druker *et al.*, 2006). The success of imatinib is also due to its 'selectivity' or rather the lack thereof. The poly-pharmacology of imatinib allowed proof of clinical concept in indications other than CML, including GIST (gastrointestinal stromal tumour), HES (hyper-eosinophilic syndrome) and others (Fabbro *et al.*, 2005). These successes convinced the pharmaceutical industry to invest in protein and lipid kinase inhibitors as targeted therapies for various cancers (Fabbro *et al.*, 2002b; 2011; Engelman, 2009; Sellers, 2011; Bartholomeusz and Gonzalez-Angulo, 2012; Workman and Al-Lazikani, 2013a; Workman *et al.*, 2013b). The sequencing of the human

kinome in 2002, the steady increase in structural analysis of protein kinases, the advent of cancer genetics in conjunction with the development of high-throughput biochemical and cell-based profiling for protein kinases led to a continuous flow of kinase inhibitor approval into the clinical space (Table 1 and Figure 1B) (Manning *et al.*, 2002; Fedorov *et al.*, 2010; Fabbro *et al.*, 2011; Workman and Al-Lazikani, 2013a; Workman *et al.*, 2013b). Thus, protein kinases have been successfully pursued since the late 1980s in the pharmaceutical industry as potential drug targets mainly for the treatment of cancer indications.

The ePK and PI3K

According to the latest counts, the human kinome contains 538 ePK genes, which are subdivided into seven families of typical and seven families of atypical protein kinases (<http://kinase.com/kinbase/>; <http://kinase.com/human/kinome/>) (Hanks and Hunter, 1995; Hunter, 2000; Manning *et al.*, 2002). The majority of ePKs are STPKs, a fact that is reflected in the ratio of cellular phosphorylation (pSer : pThr : pTyr = 1000:100:1) (Hanks and Hunter, 1995; Hunter, 2000; Cohen, 2001; 2002b; Manning *et al.*, 2002; Ubersax and Ferrell,

Table 1

Approved kinase inhibitors as of February 2015

Generic name (compound code, trade names)	Kinase target	Disease	Company (year, type)
Fasudil (HA-1077)	ROCK1/2	Cerebral vasospasm, PAH	Asahi Kasei (1995, type-1)
Sirolimus (Rapamune)	mTOR	Kidney transplants	Pfizer, Wyeth (1999, type-3)
Imatinib (STI571, Glivec, Gleevec)	ABL, PDGFR, KIT	CML, Ph+ B-ALL, CMML, HES, GIST	Novartis (2001, type-2)
Gefitinib (ZD1839, Iressa)	EGFR	NSCLC	AZ (2003, type-1)
Erlotinib (OSI-774, Tarceva)	EGFR	NSCLC, pancreatic cancer	Roche, OSI (2004, type-1)
Sorafenib (BAY 43-9006, Nexavar)	VEGFR2, PDGFR, KIT, FLT3, BRAF	RCC, HCC	Bayer, Onyx (2005, type-2)
Sunitinib (SU11248, Sutent)	VEGFR, KIT, PDGFR, RET, CSF1R, FLT3	RCC, imatinib resistant GIST	Pfizer (2006, type-1)
Lapatinib (GW2016, Tykerb)	EGFR, ERBB2	BC	GSK (2007, type-1.5)
Dasatinib (BM-354825, Sprycel)	ABL, PDGFR, KIT, SRC	CML	BMS (2007, type-1)
Nilotinib (AMN107, Tasigna)	ABL, PDGFR, KIT	CML	Novartis (2007, type-2)
Everolimus (Rad001, Certican, Zortress, Afinitor, Votubia)	mTOR	RCC, SEGA, Transplantation	Novartis (2009, type-3)
Temsirolimus (CCI-779, Torisel)	mTOR	RCC	Pfizer, Wyeth (2009, type-3)
Crizotinib (PF-02341066, Xalcori)	MET and ALK	NSCLC with ALK translocations	Pfizer (2011, type-1)
Vandetanib (ZD6474, Caprelsa)	RET, VEGFR1-2, FGFR, EGFR	MTC	AZ (2011, type-1)
Ruxolitinib (INC424, Jakafi)	JAK2	IMF with JAK2V617F mutations	Novartis, Incyte (2011, type-1)
Vemurafenib (PLX4032, RG7204, Zelboraf)	BRAF	Metastatic melanoma with BRAFV600E mutations	Roche, Plexxikon (2011, type-2)
Axitinib (AG013736, Inlyta)	VEGFR, KIT, PDGFR, RET, CSF1R, FLT3	RCC	Pfizer (2012, type-1)
Regorafenib (BAY 73-4506, Stivarga)	VEGFR2, Tie2	CRC, GIST	Bayer (2012, type-2)
Pazopanib (GW-786034, Votrient)	VEGFR, PDGFR, KIT	RCC	GSK (2012, type-1)
Tofacitinib (CP-690550, Xeljanz, Tasocitinib)	JAK3	RA	Pfizer (2012, type-1)
Cabozantinib (XL184, BMS907351, Cometriq)	VEGFR2, PDGFR, KIT, FLT3	MTC	Exelixis (2012, type-1)
Ponatinib (AP24534, Iclusig)	ABL	Imatinib resistant CML with T315I mutations	Ariad (2012, type-1)
Bosutinib (SKI-606, Bosulif)	ABL	CML resistant/ intolerant to therapy	Pfizer (2012, type-1)
Dabrafenib (Tafinlar) [6494]	BRAF	Metastatic melanoma with BRAFV600E mutations	GSK (2013, type-2)
Trametinib (Mekinist) [6495]	MEK	Metastatic melanoma with BRAFV600E mutations	GSK (2013, type-3)
Afatnib (Gilotrif, Tomtovok, Tovok)	EGFR	NSCLC with EGFR activating mutations	BI (2013, covalent)
Ibrutinib (PCI-32765, Imbruvica)	BTk	MCL, CLL	Janssen, Pharmacyclic (2013, covalent)
Ceritinib (LDK378, Zykadia)	ALK	NSCLC with ALK translocations	Novartis (2014, type-1)
Idelalisib (CAL101, GS1101, Zydelig)	PI3Kdelta	CLL, FL and SLL	Gilead, Calistoga, ICOS (2014, type-1)
Nintedanib (BIBF 1120, Vargatef, Intedanib)	VEGFR, PDGFR, FGFR	Idiopathic Pulmonary Fibrosis	BI (2014, type-1)
Alectinib (AF802, RO5424802)	ALK	ALK-rearranged NSCLC	Roche (2014)
Palbociclib (PD-0332991, Ibrance)	CDK4/6	Advanced (metastatic) BC	Pfizer (2015)
Lenvatinib (E7080)	VEGFRs	Thyroid cancer	Eisai Co (2015)

The biochemical profiles of the 33 approved kinase inhibitors are stored in the IUPHAR database (<http://www.guidetopharmacology.org/GRAC/LigandListForward?type=Approved&database=all>). The 33 kinase inhibitors approved to date are shown with generic compound name, compound code, trade name, primary indications, company and mode of binding. The approved kinase inhibitors include fasudil (HA-1077) (Shibuya and Suzuki, 1993; Shibuya *et al.*, 2001), sirolimus (Rapamycin, Rapamune®) (Kelly *et al.*, 1997; Vasquez, 2000), imatinib (Glivec®) (Druker *et al.*, 1996), gefitinib (Iressa™) (Barker *et al.*, 2001), erlotinib (Tarceva™) (Perez-Soler, 2004), lapatinib (Tykerb®) (Gaul *et al.*, 2003), sorafenib (Nexavar®) (Lowinger *et al.*, 2002), sunitinib (Sutent®) (Sun *et al.*, 2003), dasatinib (Sprycel®) (Lombardo *et al.*, 2004), nilotinib (Tasigna®) (Weisberg *et al.*, 2005), torisel (Temsirolimus®) (Galanis *et al.*, 2005), everolimus (Rad001) as Afinitor® (Chan *et al.*, 2010; Baselga *et al.*, 2012; Beck *et al.*, 2014) as Zortress® and Certican™ (Cibrik *et al.*, 2013) as Votubia® for SEGA (Krueger *et al.*, 2010), crizotinib (Xalcori™) (Shaw *et al.*, 2011), vandetanib (Caprelsa®) (Carlomagno and Santoro, 2004; Chau and Haddad, 2013), ruxolitinib (Jakafi®) (Harrison *et al.*, 2012), vemurafenib (Zelboraf®) (Flaherty *et al.*, 2010), axitinib (Inlyta®) (Ansari *et al.*, 2013; Rini *et al.*, 2013), regorafenib (Stivarga®) (Shahda and Saif, 2013), pazopanib (Votrient™) (Sternberg, 2009), tofacitinib (Xeljanz) (Simmons, 2013), cabozantinib (Cometriq) (Viola *et al.*, 2013), ponatinib (Iclusig®) (Nicolini *et al.*, 2013), bosutinib (Bosulif®) (Amsberg and Koschmieder, 2013), dabrafenib (Tafinlar®) (Ballantyne and Garnock-Jones, 2013; King *et al.*, 2013), trametinib (Mekinist®) (Salama and Kim, 2013; Wright and McCormack, 2013), afatinib (Gilotrif®) (Nelson *et al.*, 2013; Ninomiya *et al.*, 2013), ibrutinib (Imbruvica®) (McDermott and Jimeno, 2014), ceritinib (Zykadia®) (Friboulet *et al.*, 2014), idelalisib (Zydelig®) (Gopal *et al.*, 2014) and nintedanib (Vargatef®, Ofev™) (Reck *et al.*, 2014; Richeldi *et al.*, 2014), alectinib (Yang, 2013), palbociclib (Ibrance®) (<http://www.onclive.com/web-exclusives/FDA-Approves-Palbociclib-for-Metastatic-Breast-Cancer>) and lenvatinib (<http://www.eisai.com/news/enews201407pdf.pdf>). All compounds are commercially available. AZ, Astra-Zeneca; BI, Boehringer-Ingelheim; GSK, Glaxo-Wellcome.

CLL, chronic lymphocytic leukaemia; CML, chronic myeloid leukaemia; CMML, chronic myeloid monocytic leukaemia; CSF1R, colony stimulating factor 1 receptor; FL, follicular lymphoma; HCC, hepatocellular cancer; IMF, idiopathic myelofibrosis; MCL, mantle cell lymphoma; MTC, medullary thyroid cancer; NSCLC, non-small-cell lung cancer; PAH, pulmonary arterial hypertension; RCC, renal cell carcinoma.

2007). Although only a minor number of substrates are phosphorylated by TPKs, the importance of tyrosine phosphorylation is demonstrated by the many gain of function (GOF) and/or loss of function (LOF) mutations that are found in TPKs (Hunter, 2000; Blume-Jensen and Hunter, 2001; Cohen, 2001; 2002b; Greenman *et al.*, 2007; Thomas *et al.*, 2007; Fedorov *et al.*, 2010; Lahiry *et al.*, 2010; Fabbro *et al.*, 2011; Workman *et al.*, 2013b). TPKs can be subdivided into two main classes (receptor TPKs and non-receptor TPKs). In contrast, the STPKs are a more heterogeneous class of enzymes and are divided into six main families of typical ePKs, including the TKL (the Tyrosine Kinase Like group closely related to the TPKs), the CMGC (the cyclin-dependent kinases, MAP kinases, Glycogen synthase kinases, Casein kinases 2), the AGC (PKA, PKG and PKC), the CAMK (CALcium/calMODulin-dependent Kinases), the STE20 [homologues of yeast Sterile 7, Sterile 11, STerile 20 kinases which include the MAP2Ks (mitogen activated kinase kinase), MAP3Ks (mitogen activated kinase kinase kinase) and MAP4Ks (mitogen activated kinase kinase kinase kinase)] and finally the CK1 (Casein Kinases 1). Most of the atypical ePKs are STPKs, which indicates that the ePK domain phylogeny may reflect substrate specificity and/or mode of regulation (<http://kinase.com/kinbase/>) (Manning *et al.*, 2002). About 10% of the human protein kinases are so-called pseudo-kinases because they are either only weakly active or presumed to be inactive. These pseudo-kinases are evenly distributed over the human kinome (Boudeau *et al.*, 2006; Kannan and Taylor, 2008). They lack at least one of three motifs in the catalytic domain that are essential for catalysis (Figure 2B). Although the non-catalytic functions are poorly understood, the pseudo-kinases can bind ATP and appear to have important regulatory functions as exemplified by the regulation of the LKB1 (serine/threonine-protein kinase STK11) by the pseudo-kinase STRAD1 (STE20-related adapter alpha) or the 'activation' of Janus kinase 2 (JAK2) catalytic domain (JH1) via a single amino acid substitution (V617F) in its JH2 (JAK homology domain-2)-pseudo-kinase domain (Kralovics *et al.*, 2005; Boudeau *et al.*, 2006; Kannan and Taylor, 2008; Zeqiraj *et al.*, 2009; Rajakulendran and Sicheri, 2010).

Another important set of kinases that play essential roles in the eukaryotic signalling and which share the protein kinase-like (PKL) fold with the ePKs include the phosphatidyl-inositol (PI) kinases and related protein kinases (Kannan *et al.*, 2007). In particular, the PI3Ks, which phosphorylate PI together with the atypical STPK mTOR, have been implicated in cancer and immunological disorders (Engelman, 2009; Courtney *et al.*, 2010; Rommel, 2010; Fruman and Rommel, 2014).

The physiological activation of kinase occurs in many different ways and their mechanisms of activation have been summarized in excellent reviews (Taylor *et al.*, 2005; Murray, 2007; Rommel *et al.*, 2007; Schmierer and Hill, 2007; Engelman, 2009; Malumbres and Barbacid, 2009; Kawai and Akira, 2010; Lemmon and Schlessinger, 2010; Mendoza *et al.*, 2011; Hoesel and Schmid, 2013; Brooks *et al.*, 2014; Hardie, 2014). Kinases are organized in cascades, which are typically initiated by various receptors including receptor and non-receptor TPKs or STPKs, which further pass their signals through various downstream effectors such as the PI3K/mTOR, the RAS-RAF-MAPK, the SMAD

[composite of MAD from drosophila Mothers Against Decapentaplegic and SMA of *Caenorhabditis elegans* (from gene *sma* for small body size)], the STAT to the cell cycle kinases and kinases regulating transcription (Lahiry *et al.*, 2010; Lemmon and Schlessinger, 2010). Besides transferring the gamma phosphate of ATP onto hydroxyl groups of substrates protein and lipid kinase, protein kinases also utilize non-catalytic functions for scaffolding, relocation, allosteric effects, subcellular targeting, DNA binding as well as protein-protein interactions (Rauch *et al.*, 2011). Abnormal hyperactivity, due to mutations, chromosomal rearrangements and/or gene amplification or LOFs of protein and PI3K kinases, plays a role in a wide variety of diseases, including cancer, inflammatory diseases, diabetes, atherosclerosis and immunological disorders (Blume-Jensen and Hunter, 2001; Cohen, 2001; Chico *et al.*, 2009; Lahiry *et al.*, 2010; Muller and Knapp, 2010; Rommel, 2010; Fabbro *et al.*, 2011; Angulo *et al.*, 2013). One-third of all protein targets under investigation in the pharmaceutical industry are protein or PI3K kinases, although their potential has so far not been fully exploited (Fedorov *et al.*, 2010). In summary, at present, a set of divergent protein and PI3Ks represent an important class of enzymes for treating human disorders.

Approved protein kinase inhibitors to date

Since the approval of fasudil in 1995, the number of approved kinase inhibitors has increased to 33 with many others still in preclinical development (Figure 1B and Table 1). More than 130 kinase inhibitors are reported to be in Phase-2/3 clinical trials (Vieth *et al.*, 2005) (<http://www.clinicaltrials.gov/>) (<http://chembl.blogspot.ch/2013/09/the-clinical-kinome-in-2013.html>). It is beyond the scope of this review to discuss all the protein kinase inhibitors that are in preclinical or in early clinical development. It should be emphasized that all of the mentioned approved and clinically advanced kinase inhibitors (Phase-3) with a few exceptions, like the rapalogs and trametinib, are directed towards the ATP binding site and do not cover more than 20% of the whole kinome (Fedorov *et al.*, 2010).

Most of the approved kinase drugs are active against more than one type of cancer. Only a few of them have been used for the treatment of non-oncological indications, namely tofacitinib for rheumatoid arthritis, sirolimus for organ rejection, fasudil for cerebral vasospasm and more recently nintedanib for idiopathic pulmonary fibrosis (Table 1, <http://www.discoverx.com/tools-resources/interaction-maps>). In contrast, there are numerous kinase drugs for one single indication. For example, imatinib, nilotinib, dasatinib, bosutinib and ponatinib have all been approved for CML, whereas sorafenib, sunitinib, everolimus, temsirolimus, axitinib or pazopanib are indicated for various stages of renal cell cancer. Ceritinib, crizotinib and alectinib are used for the treatment of non-small-cell lung cancer (NSCLC) with anaplastic lymphoma kinase (ALK) translocations, while gefitinib, erlotinib and afatinib are indicated for NSCLC with activated EGFR. Vandetanib, cabozantinib and levatinib are used for the treatment for medullary thyroid carcinoma, while imatinib,

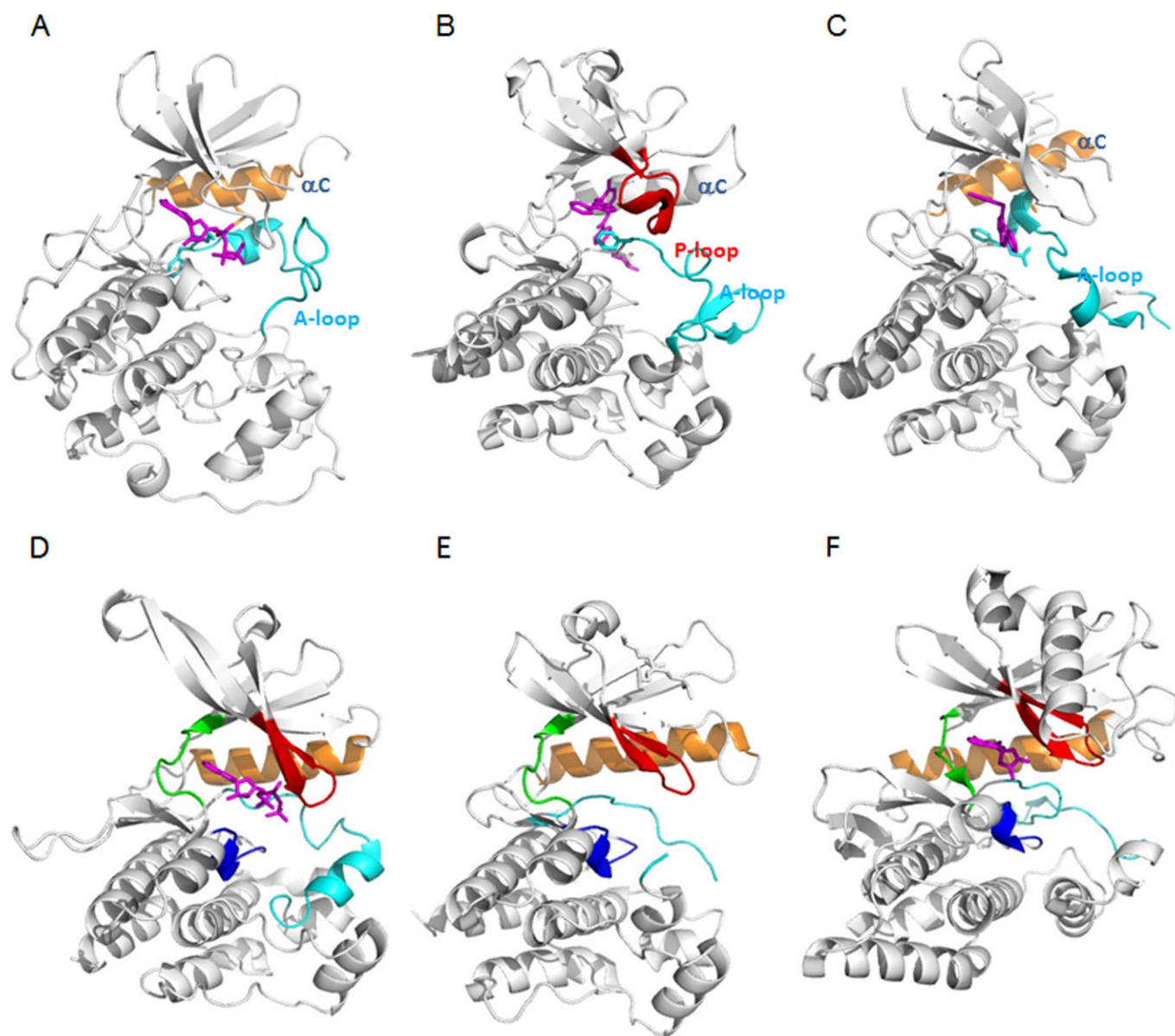


Figure 2

Kinase (inactive), pseudokinase and atypical kinase conformational states. Various examples of the positions and forms of structural elements in different kinases. The same colour scheme as in Figure 2 is used. Panels (A)–(C) show the inactive states of three kinases, with only those structural elements where there are large shifts compared with the active state coloured. (A) Inactive conformation of Hck: C-helix out and closed A-loop (PDB entry 1HCK). (B) Inactive conformation of Abl1 kinase: Collapsed P-loop and closed A-loop with DFG-motif out (PDB entry 1IEP). (C) Inactive conformation of c-Met: C-helix out and yet another conformation of the A-loop (PDB entry 3CCN). Panels (D) and (E) show the structures of the pseudokinases JAK2 (PDB entry 4FVR), where the HRD sequence motif is not conserved, and MLKL (PDB entry 4MWI), where both the DFG and HRD sequence motifs are not conserved respectively. Panel (F) shows the structure of the atypical kinase, haspin (PDB entry 2VUW).

sunitinib and regorafenib are indicated also for GIST. Finally, vemurafenib or dabrafenib in combination with trametinib is indicated for metastatic melanoma with BRAFV600 mutations (Table 1).

The conservation of the ATP binding site in the human kinome often causes these 'ATP-mimetics' to cross-react with many other different kinases, resulting in compounds with promiscuous profiles. Promiscuous compounds like, for example, dasatinib (Lombardo *et al.*, 2004) or sunitinib

(Motzer *et al.*, 2006; Faivre *et al.*, 2007) have been termed multi-kinase inhibitors but have some toxicological liabilities (Cheng and Force, 2010). In contrast, kinase inhibitors targeting the ATP site, such as lapatinib, tofacitinib or imatinib, are reasonably selective (<http://www.discoverx.com/tools-resources/interaction-maps>).

These ATP-site-directed inhibitors may be viewed as first generation, as they have demonstrated appropriate selectivity, potency and pharmacokinetic (PK) properties. However,

the usually poor physicochemical properties, the limited selectivity and the relatively restricted ATP pharmacophore with an extensive coverage of chemo-types remain as the main challenges for kinase drug discovery (Traxler *et al.*, 2001; Cowan-Jacob, 2006; Engelman, 2009; Zhang *et al.*, 2009; Fabbro *et al.*, 2011; Liu *et al.*, 2013). We are just beginning to have a molecular and structural understanding of the regulation of the kinase activity, both at the level of the kinase domain as well as at the level of the full-length protein kinases. There is now increasing interest in identifying inhibitors that do not compete with ATP. Kinase inhibitors with outstanding selectivity are likely to become important not only for minimizing side effects and allowing chronic treatment of non-life-threatening diseases, but also to better understand the on- and off-target pharmacology of kinase inhibitors (Robert *et al.*, 2005; Force *et al.*, 2007; Fabbro *et al.*, 2011; Moebitz and Fabbro, 2012; Cowan-Jacob *et al.*, 2014).

While the mutational status of kinases may be associated with various cancer conditions, the identification and validation of the driver kinase(s) in these diseases by genome-wide screening for kinase amplifications, translocations and/or mutations as well as studying the multiple mechanisms of resistance is an area of intense research to improve the efficacy of these targeted therapies (Hunter, 2000; Blume-Jensen and Hunter, 2001; Cohen, 2002b; Weinstein, 2002; Bardelli *et al.*, 2003; Sawyers, 2004; Vieth *et al.*, 2004; Takano *et al.*, 2005; Ventura and Nebreda, 2006; Wolf-Yadlin *et al.*, 2006; Ali and Ali, 2007; Engelman *et al.*, 2007; Greenman *et al.*, 2007; Thomas *et al.*, 2007; Luo *et al.*, 2009; Stransky *et al.*, 2014).

In conclusion, the actual landscape of kinase inhibitor drugs developed over the last two decades shows that

- only a small number of protein and lipid kinase targets (about 80) out of the 500+ protein kinases in the human kinome have been successfully targeted
- most of the kinase inhibitor drugs are used for oncological indications
- many kinase inhibitor drugs are used to target the same indication (mainly due to the generation of resistance)

The structure and catalytic mechanisms of ePKs

The ePK protein kinase domain has evolved to have many different regulatory mechanisms and is often associated with a large variety of other protein domains that directly or indirectly contribute to the regulation of the kinase activity (Nolen *et al.*, 2004; Cowan-Jacob *et al.*, 2009; 2014; Scott and Pawson, 2009; Zhang *et al.*, 2009; Taylor and Kornev, 2011; Jin and Pawson, 2012). The ePKs and ELKs share the PKL-fold and similar catalytic mechanisms, although ELKs generally display very low sequence identity with ePKs and with each other (Kannan *et al.*, 2007). The overall structural organization of the ca. 300 residue protein kinase domain is conserved with 10 key residues mediating the core functions of the catalytic domain (Hanks *et al.*, 1988; Manning *et al.*, 2002; Kannan *et al.*, 2007). All the other structural elements

outside the kinase domains typically serve either as regulatory or as targeting modules (Scott and Pawson, 2009; Jin and Pawson, 2012).

All protein kinase domains consist of a small, mostly β -stranded N-lobe, connected by a short hinge region to a larger α -helical C-lobe (Figure 3). ePKs bind the ATP in the cleft between the N- and C-terminal lobes of the kinase domain where the adenine group of ATP is sandwiched between hydrophobic residues and makes contact via hydrogen bonds to the hinge region (Figure 3A and B) (Nolen *et al.*, 2004; Taylor and Kornev, 2011; Cowan-Jacob *et al.*, 2014).

The N-lobe contains a five-stranded β -sheet (β 1– β 5) with a single α -helix (the C-helix, α C). The Gly-rich loop (also known as P-loop or G-loop) lies between the β 1 and β 2 strands and contains an important hydrophobic residue at its tip, which contributes to coordination of the phosphates of ATP (Figure 3) (Nolen *et al.*, 2004; Cowan-Jacob, 2006; Taylor and Kornev, 2011). This is the most flexible part of the N-lobe, which folds over the nucleotide positioning the γ -phosphate of ATP for catalysis. The C-terminus of the C-helix is anchored to the core of the C-lobe by the β 4-loop via the β 5 strand, which continues into the hinge region, whereas its N-terminus interfaces with the activation loop (also called activation segment or A-loop). The A-loop occurs either in an open (the hallmark for the active ATP-bound state of the kinase) or various closed conformations, indicating the inactive state of the kinase by occluding the access of the protein substrate sites (Figure 3) (Nolen *et al.*, 2004; Cowan-Jacob, 2006). The N-terminus of the C-helix has to be positioned correctly for efficient catalysis facilitating the interaction between the active site Lys (of the AXK-motif in the β 3-strand) and the Glu from the C-helix ('C-helix-in'). Rotating the N-terminus of the C-helix in a suboptimal position for catalysis ('C-helix-out') results in an inactive state of the kinase (Cowan-Jacob, 2006; Kannan *et al.*, 2007; Taylor and Kornev, 2011; Moebitz and Fabbro, 2012). N-terminal to the hinge, deep in the ATP pocket, is an important residue called the 'gatekeeper', which controls the access to the 'back-pocket' of the kinase and which is often mutated in kinases resistant to inhibitors (Figure 3C) (Nolen *et al.*, 2004; Kornev *et al.*, 2006; Cowan-Jacob *et al.*, 2009; Taylor and Kornev, 2011; Moebitz and Fabbro, 2012).

The larger lobe or C-terminal lobe of the kinase domain is mostly helical. There are four β -strands in the active state: β 6 and β 7 contain the catalytic loop with most of the catalytic machinery (Y/HRD or Tyr/His-Arg-Asp), whereas β 8 and β 9 flank the DFG-motif where the Asp recognizes one of the ATP-bound Mg^{2+} . The Phe of the DFG-motif (Aspartate-Glycine-Phenylalanine or Asp-Gly-Phe) makes hydrophobic contacts with the C-helix and the nearby Y/HRD-motif from the catalytic loop (Figure 3). The Asp of the Y/HRD, one of the most conserved residues (present in all ePKs), is responsible for correct orientation of the P-site hydroxyl acceptor group in the peptide substrate. Similarly, the Tyr/His in Y/HRD is conserved throughout all ePKs and ELKs and serves as a central scaffold for binding both to the carbonyl group of Asp and making a hydrophobic contact to the Phe of the DFG-motif. The Mg^{2+} -binding loop, which is followed by the β 9 strand, forms an antiparallel β -sheet with the β 6 strand that precedes the catalytic loop (Y/HRD-motif). This portion of the sheet is disordered in the inactive kinases and is

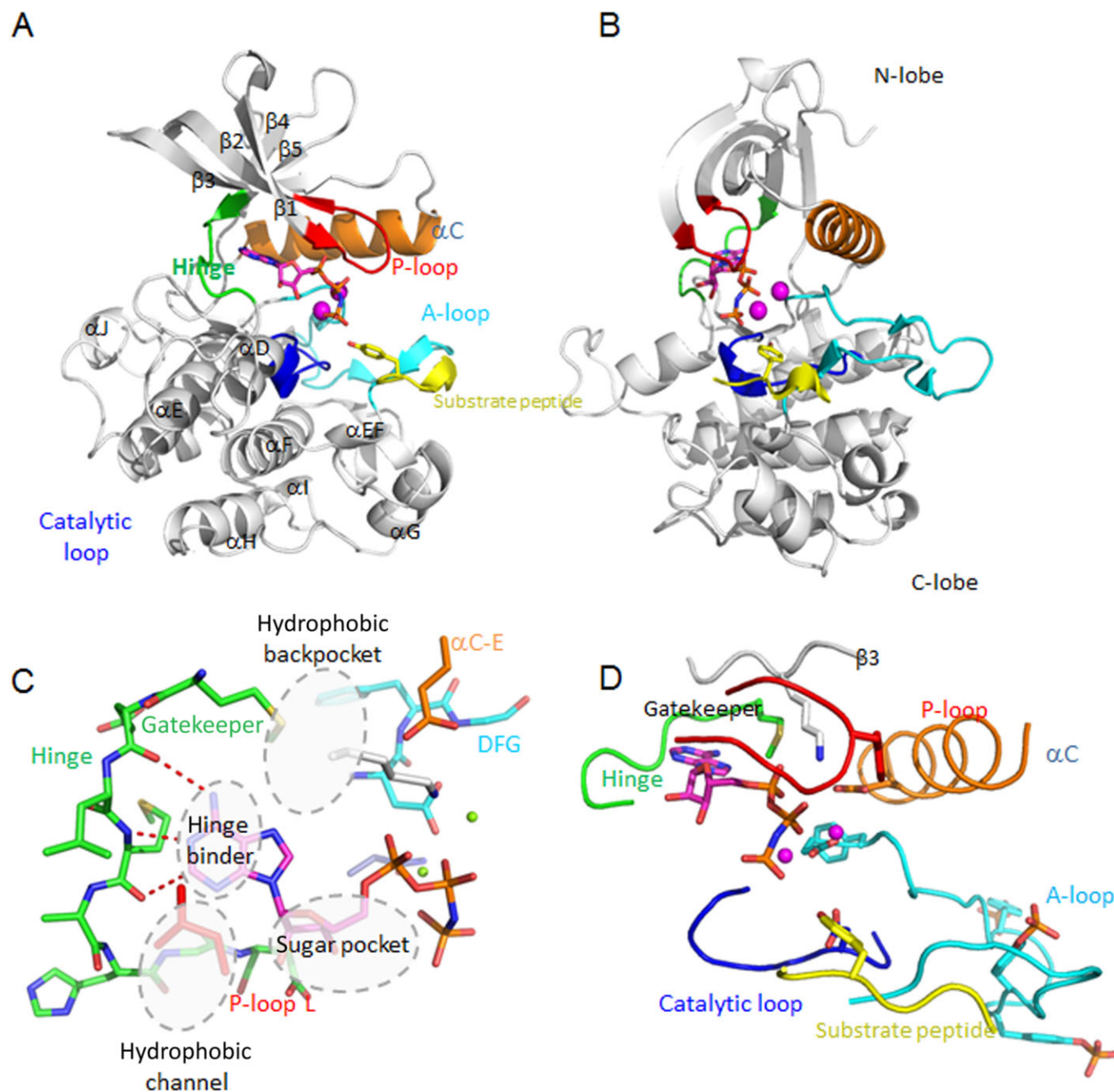


Figure 3

The active conformation of protein kinases. Front (A) and side views (B) of a typical active kinase conformation displaying the ternary complex of the insulin receptor (InsR), ATP and peptide substrate (pdb 1ir3). The helices and β -sheets forming the canonical kinase fold are labelled, as well as important secondary structure elements which are shown colour coded. (C) The ATP-site pharmacophore: The hydrophobic channel, the sugar pocket, the hinge and the hydrophobic back-pocket are the major pharmacophores. (D) Close-up of the active ATP site of the InsR. For explanations, see text. The two Mg^{2+} ions are in magenta.

believed to be important for the correct Mg^{2+} -binding loop configuration. The A-loop, which includes the $\beta 9$ strand, extends from the DFG-motif (at the very N-terminus of the A-loop) to a conserved Asp at the beginning of the F-helix. The Phe of the DFG-motif is responsible for proper positioning of the Asp and accommodation of the C-helix facilitating the Lys–Glu salt bridge. The flexible A-loop regulates the on

and off state of the kinase by providing the platform together with the helical subdomains of the C-lobe for binding and positioning of the hydroxyl group residue of the peptide substrate (Nolen *et al.*, 2004; Ubersax and Ferrell, 2007). The extended helical element that follows the F-helix is unique to the ePKs and includes the G-helix through the I-helix (GHI domain). Many substrate proteins and regulatory proteins are

tethered to the GHI domain (Figure 3A) (Taylor and Kornev, 2011).

In summary, three sequence motifs are essential for catalysis (Hanks and Hunter, 1995; Cowan-Jacob, 2006; Taylor and Kornev, 2011):

- 1 The AXK-motif ($\beta 3$ strand) with the active site Lys forming a salt bridge with the conserved Glu from the C-helix, which interact with the α and β phosphates of ATP to anchor and orient the ATP
- 2 The Y/HRD-motif or catalytic loop ($\beta 6/\beta 7$), in which the Asp is the catalytic residue functioning as a base acceptor for the proton transfer
- 3 The DFG-motif of the A-loop where the Asp binds the Mg^{2+} ions that coordinate the β and γ phosphates of ATP in the ATP binding cleft positioning the latter for the phosphate transfer

Activation of protein kinases results in the re-orientation of the C-helix ('C-helix-in') to bring the conserved Glu into the proximity of the active site Lys of the AXK-motif as well as the A-loop. In many instances, the Phe of DFG moves from the DFG-out (an inactive conformation) into the DFG-in position (usually an active conformation) (Figure 2). The extended β -sheet conformation of the G-loop helps coordinating the phosphates of ATP, whereas the $\beta 6$ strand forms part of the catalytic loop that facilitates the phosphor transfer. The catalytic loop (Y/HRD-motif) is the only conserved element that does not differ between the active and inactive states of the protein kinase. The short EF-helix at the end of the A-loop with the conserved Glu of the APE-motif forms the peptide substrate binding site. The A-loop in the active conformation can be stabilized by phosphorylation or interactions with accessory regulatory proteins (Figure 2) (Nolen *et al.*, 2004; Cowan-Jacob *et al.*, 2009).

The regulation of the catalytic mechanism of protein kinases may further involve a regulatory (R) and a catalytic (C) spines, which are each built up by two conserved residues from and the N- and C-lobes respectively. The R-spine is formed by four hydrophobic residues, one from the $\beta 4$ strand, one from the C-helix, the Phe from the DFG and the Tyr/His from the catalytic loop. Proper alignment of these hydrophobic residues results in the formation of the R-spine linking the N- and C-lobes for optimal protein kinase activity. The C-spine comprises two residues from both lobes and is completed by the adenine ring of ATP. The Val in the $\beta 2$ strand and the Ala from the AXK-motif of the $\beta 3$ strand are docked directly onto the adenine ring of ATP. The hydrophobic residue lies in the middle of $\beta 7$ strand of the C-lobe and which, on the one hand, docks directly onto the adenine ring and, on the other hand, rests on a hydrophobic residue from the D-helix, which, in turn, is bound to the F-helix (Kornev *et al.*, 2006; Taylor and Kornev, 2011).

The different modes to inhibit kinases

Low-molecular-weight kinase inhibitors can bind either covalently or reversibly to kinases (Zhang *et al.*, 2009; Liu *et al.*, 2013; Cowan-Jacob *et al.*, 2014).

Covalent inhibitors

Covalent inhibitors usually have a binding, a linker and a warhead module that can bind in or close to the ATP binding sites. Depending upon the reactivity of the warhead, the covalent binding can be reversible (Wymann *et al.*, 1996; Liu *et al.*, 2013). It should be mentioned that drugs that bind covalently to their targets have always been perceived as being potentially toxic. However, it should be emphasized that many marketed drugs bind covalently to their targets (Singh *et al.* 2011). Covalent kinase inhibitors usually target the active site Lys or a Cys in or around the ATP binding site (Figure 4D) (Wymann *et al.*, 1996; Rabindran *et al.*, 2004; Kwak *et al.*, 2005; Zhou *et al.*, 2009). Various covalent kinase inhibitors have been identified for various protein kinases, including Fes (Feline sarcoma oncogene kinase) (Filippakopoulos *et al.*, 2008), VEGFR-2 (Wissner *et al.*, 2007), ribosomal S6 kinase (Cohen *et al.*, 2007) and Bruton tyrosine kinase (BTK) (Pan *et al.*, 2007). Some of them have progressed into the clinic like AVL-292, an orally available, selective covalent inhibitor of BTK that is currently undergoing Phase-2 clinical trials for chronic lymphocytic leukaemia and non-Hodgkin lymphoma (Robak and Robak, 2012). Other covalent inhibitors like ibrutinib, targeting BTK, and afatinib, targeting the gefitinib-resistant EGFR, have been recently approved by the Food and Drug Administration (Figure 4D and Table 1) (Minkovsky and Berezov, 2008; Ninomiya *et al.*, 2013; Akinleye *et al.*, 2014). Although these

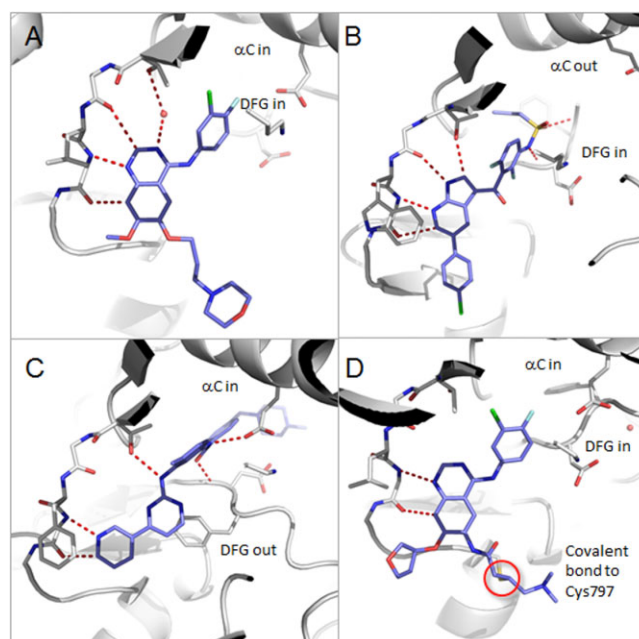


Figure 4

Representative binding modes of the four classes of kinase inhibitors. Representative binding modes of the four classes of kinase inhibitors with ligand in blue sticks and key polar interactions shown as red dotted lines: (A) gefitinib bound to EGFR (type 1, pdb 2ity); (B) vemurafenib bound to B-Raf (type 1.5, pdb 3og7); (C) imatinib bound to ABL (type 2, pdb 1iep); and (D) afatinib bound covalently to EGFR (pdb 4g5j). Activation states of helix-C and the DFG-motif are annotated.

covalent kinase inhibitors have shown impressive clinical results, it should be mentioned that mutation of the Cys, which is not essential for the kinase structure and activity, can prevent the covalent binding and their clinical efficacy (Furman *et al.*, 2014).

Non-covalent inhibitors

The non-covalent kinase inhibitors can be further classified into those that either bind or do not bind to the hinge region of the kinase (Figure 3C), leading to the classification of type-1, type-2 and type-3 reversible kinase inhibitors (Traxler *et al.*, 2001; Li *et al.*, 2004; Liu and Gray, 2006; Cowan-Jacob *et al.*, 2009; Zhang *et al.*, 2009; Moebitz and Fabbro, 2012).

Type-1 and type-1.5 inhibitors. The vast majority of the ATP-competitive inhibitors bind to active conformations with the conserved Phe residue of the DFG-motif buried in a hydrophobic pocket in the groove between the two lobes of the kinase (Figures 3 and 4A and B) (Pargellis *et al.*, 2002; Li *et al.*, 2004; Liu and Gray, 2006; Cowan-Jacob *et al.*, 2009; Zhang *et al.*, 2009; Moebitz and Fabbro, 2012). The ATP binding site of active protein kinases (and PI3Ks) is very similar, despite the fact that they have different substrate specificities and different modes of regulation. In the active conformation, the A-loop adopts an open conformation typical for the ATP-bound state of the kinase where the Asp in the DFG-motif coordinates the phosphates of ATP, whereas the Phe stabilizes the C-helix and the A-loop for catalysis (Figure 3D) (Nolen *et al.*, 2004; Cowan-Jacob, 2006; Liu and Gray, 2006; Zhang *et al.*, 2009; Cowan-Jacob *et al.*, 2014). Type-1 inhibitors utilize variation in the size, shape and polarity of the gatekeeper residue to gain selectivity (Figure 3C). Finding compounds that target the active conformation of the kinase by ATP mimetics is best achieved using enzymatic kinase assays displaying the highest level of activity. Classical examples for this type of approved kinase inhibitor class are gefitinib, erlotinib, dasatinib and sunitinib (Table 1). The type-1.5 inhibitor, exemplified by vemurafenib, is a subtype of the type-1 inhibitor that binds to an inactive kinase conformation (Figure 4B) (Tsai *et al.*, 2008; Zuccotto *et al.*, 2010). In this case, the BRAF adopts a DFG-in conformation, typical of an active kinase, but with the C-helix being pushed out ('C-helix-out') by vemurafenib effectively disrupting the ion pairing between the active site Lys and the Glu from the C-helix (Tsai *et al.*, 2008). This type-1.5 inhibitor with a 'DFG-in' inactive conformation has also been observed in other kinases (Figure 2A and C) (Cowan-Jacob *et al.*, 2014).

Selective type-1 or type-1.5 inhibitors use additional sites close to the ATP binding site, like the adjacent hydrophobic pockets (Figure 3C) whose entry is regulated by the gatekeeper (Zuccotto *et al.*, 2010), or additional sites close to the peptide binding site, like the bivalent/bitopic inhibitors (Hill *et al.*, 2012), the macrocycles (Tao *et al.*, 2007) or some of the covalent inhibitors (Liu *et al.*, 2013). The success of type-1 inhibitors in the clinic demonstrates that, despite the highly conserved ATP binding site, it is feasible to optimize selectivity for kinases by following appropriate strategies, which is reflected in the fact they represent the vast majority of the kinase inhibitors. The most rational way to obtain selectivity is by targeting poorly conserved residues, particularly resi-

dues flanking the hinge. Although typically discovered by serendipity rather than rational design, the interplay of sequence and conformational penalty can lead to exceptional selectivity. The balance between sequence and conformational contributions comes in different flavours. In one extreme, optimization of the compound leads from an active to an inactive, high-energy conformation of the kinase such that the additional interactions make up for the conformational penalty, but only on the target kinase. Examples are vemurafenib ('C-helix-out' and 'DFG-in', type-1.5 inhibitor) versus the relatively unselective type-1 inhibitors gefitinib (Figure 4A and B) (Wood *et al.*, 2004). Another example is the MET (mesenchymal epithelial transition factor or hepatocyte growth or scatter factor receptor) kinase whose native, inactive conformation offers the potential for a unique, crucial stacking interaction with a Tyr in the A-loop where several unique sequence features play together to stabilize an otherwise high-energy conformation (Figure 2C) (Albrecht *et al.*, 2008).

The ability of the G-loop of kinases to partially collapse onto the ligand, thus creating a more buried, less solvent-exposed cavity with high intrinsic ligand efficiency, is another element for selectivity like in ABL (Figure 5B), which include other kinases that show high intrinsic ligand efficiency like the Aurora kinase and GSK3 β (glycogen synthase kinase-3 β). Lastly, there are cases of excellent selectivity, which arise from a multitude of subtle interactions as exemplified by the JAK inhibitor CP-690,550, which, again, builds upon a standard, pan-kinase-inhibitor-pyrrolo-pyrimidine scaffold (West, 2009; Williams *et al.*, 2009) (<http://www.discoverx.com/tools-resources/interaction-maps>).

The type-2 inhibitors. The type-2 kinase inhibitors preferentially bind to the inactive conformation of the protein kinase and still have contact with the hinge (Liu and Gray, 2006; Cowan-Jacob *et al.*, 2009; Zhang *et al.*, 2009). They usually score as ATP-competitive and bind to the inactive, the so-called 'DFG-out', conformation (Figure 4C) (Nolen *et al.*, 2004; Cowan-Jacob, 2006; Liu and Gray, 2006; Zhang *et al.*, 2009). The transition from the 'DFG-in' to the 'DFG-out' conformation exposes an additional hydrophobic pocket adjacent to the ATP site that is utilized by type-2 inhibitors locking the kinase in the inactive conformation (Nolen *et al.*, 2004; Cowan-Jacob, 2006; Liu and Gray, 2006; Zhang *et al.*, 2009; Cowan-Jacob *et al.*, 2014).

Type-2 inhibitors are in general less promiscuous than type-1 inhibitors as revealed by several selectivity profiles. Although some type-1 inhibitors can be very specific, there are also examples of type-2 inhibitors that are rather promiscuous (Goldstein *et al.*, 2008; Karaman *et al.*, 2008; Anastassiadis *et al.*, 2011). Approved kinase inhibitors binding to or stabilizing the 'DFG-out' conformations are imatinib, nilotinib or sorafenib (Table 1).

In addition to the DFG-out, combinations of different conformational states of C-helix, the A- and/or the P-loop can generate various inactive conformations of the kinase domain (Cowan-Jacob, 2006; Cowan-Jacob *et al.*, 2014). Each individual kinase has a preferred inactive conformation, depending upon its phosphorylation state and regulatory mechanisms involving structures outside the kinase domain (Cowan-Jacob, 2006; Cowan-Jacob *et al.*, 2014).

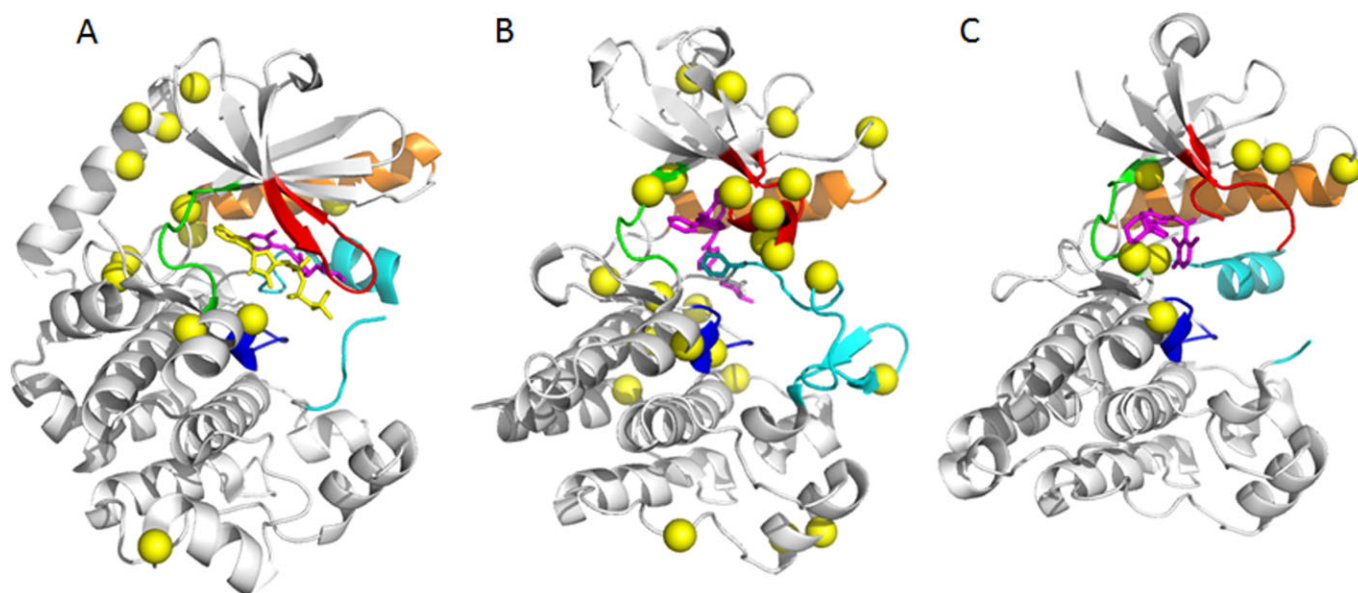


Figure 5

Clinically relevant resistance mutations of MEK1, ABL1 and ALK. (A) MEK1 and MEK2 mapped onto pdb 3eqc. The majority of the mutations cluster at the interface with the autoinhibitory N-terminal helix (top left in this view). (B) ABL mapped onto pdb 1iep. The imatinib-resistant mutants are spread all over the kinase domain; however, the most resistant against imatinib is the gatekeeper mutant T315I in the hinge (green) and the mutations located in the P-loop (red). (C) ALK mapped onto pdb 2xp2. Crizotinib-resistant mutations. The most resistant mutation is L1196M in the hinge (green) region.

Another interesting mechanism of inhibition that requires an ATP-site-directed kinase inhibitor, irrespective of its type, concerns the interactions of the molecular chaperone HSP90–CDC37 system and mutated versions of kinases. CDC37 appears to inhibit the binding of ATP to the kinases they regulate. This interaction can be inhibited with ATP-site-directed kinase inhibitors, thereby destabilizing the influence of the HSP90–CDC37 chaperone system on the kinase, resulting in the destruction of the kinase through an HSP90-independent degradation pathway (Polier *et al.*, 2013).

Type-3 (allosteric) inhibitors. The type-3 inhibitors are a heterogeneous group of kinase inhibitors that bind to allosteric or remote sites on the kinase and include, for example, inhibitors targeting MEK1 (mitogen activated kinase kinase-1), CHEK1 (checkpoint kinase-1), ABL, FAK (focal adhesion kinase) or Akt (protein kinase B or kinase from the transforming oncogene AKT8) (Figure 6) (Ohren *et al.*, 2004; Barnett *et al.*, 2005; Lindsley *et al.*, 2005; Adrian *et al.*, 2006; Converso *et al.*, 2009; Vanderpool *et al.*, 2009; Wang and Sun, 2009). The type-3 inhibitors are non-ATP site (allosteric) kinase inhibitors that have no physical contact with the hinge and show the highest degree of selectivity by exploiting binding sites and regulatory mechanisms that are unique to a particular kinase (McIntyre *et al.*, 2003; Ohren *et al.*, 2004; Barnett *et al.*, 2005; Lindsley *et al.*, 2005; Adrian *et al.*, 2006; Converso *et al.*, 2009; Cowan-Jacob *et al.*, 2009; Vanderpool *et al.*, 2009; Wang and Sun, 2009; Zhang *et al.*, 2009; Fabbro *et al.*, 2012). While most type-3 inhibitors are non-ATP-competitive or ATP-uncompetitive, some compete with ATP indirectly by binding to mutually exclusive conformations. The non-catalytic roles of kinases involve unique

non-conserved interactions and increase the target space on the kinome (Rauch *et al.*, 2011; Cowan-Jacob *et al.*, 2014). In addition to the 'DFG-in' and 'DFG-out' combinations of different states of the C-helix, the A-loop and/or the G-loop can generate various inactive conformations of the kinase domain. Moreover, elements outside the kinase domain like the juxta-membrane region of the receptor PTKs or other N- or C-terminal elements, linkers and/or other regulatory domains required for protein–protein interactions are all important elements in the regulation of the catalytic domain (Cowan-Jacob *et al.*, 2009; Zhang *et al.*, 2009; Fabbro *et al.*, 2012; Moebitz and Fabbro, 2012). The unique combinations of all these structural elements create a structural diversity that can be used to design selective inhibitors with clear advantages over the regular type-1 and type-2 ATP site inhibitors. This includes improved selectivity and slower off-rates, which increase the residence time of the inhibitor bound to the kinase (Wood *et al.*, 2004; Tummino and Copeland, 2008). However, the paucity of available structures for the inactive protein kinase (apo-form), along with the lack of a general method to assay for such inhibitors, represents a major difficulty in identifying inhibitors targeting the inactive conformations. Type-3 inhibitors can bind either to the kinase domain (close to or removed from the ATP site) or to sites that are located outside the kinase domain.

The type-3 inhibitors include very diverse compounds ranging from the MEK1 inhibitors to rapamycin derivatives. For example, the allosteric type-3 inhibitors of MEK1 bind to a pocket adjacent to the ATP binding site, referred to as 'allosteric back-pocket' (Ohren *et al.*, 2004), in the presence of ATP and are referred to as 'allosteric back-pocket-DFG-in' inhibitors (Figure 6). Other type-3 inhibitors bind to the

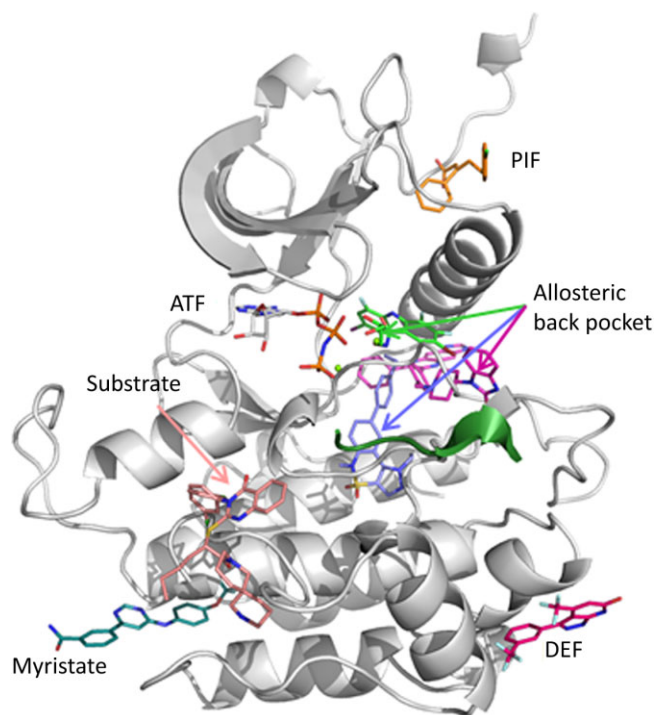


Figure 6

Allosteric pockets. Examples of allosteric ligands mapped onto an active kinase conformation, comprising the myristate site of ABL (pdb 3k5v), the PIF pocket in PDK1 (3hrf), the substrate site in CHK1 (pdb 3f9n), the DEF (docking site for ERK) site in p38 (pdb 3new) and the allosteric back-pocket in MEK1 (DFG-in, pdb 1s9j, green), Akt1 (DFG-out, pdb 3o96, magenta) and FAK (DFG-out, pdb 4ebw, blue).

'allosteric back-pocket' in the absence of ATP in the 'DFG-out' conformation like the IGF1R (Heinrich *et al.*, 2010), FAK (Tomita *et al.*, 2013) or p38 (Over *et al.*, 2013) and are referred to as 'allosteric back-pocket-DFG-out' inhibitors (Figure 6). In the case of IGF1R, the inhibitor binds the 'allosteric back-pocket' and extends over towards the substrate binding site and the catalytic loop. In contrast, the type-3 FAK inhibitors extend from the 'allosteric back-pocket-DFG-out' into a pocket formed in the C-lobe by displacing the normally rigid catalytic loop (Tomita *et al.*, 2013). The allosteric Akt inhibitors are a special case of the 'allosteric back-pocket-DFG-out' as they only bind to this site when the pleckstrin homology domain of Akt is present (Figure 6). Therefore, their identification required the full-length protein for the kinase assay (Barnett *et al.*, 2005; Lindsley *et al.*, 2005). While lack of competition with ATP has, in some cases, proven to be a useful way to identify type-3 inhibitors, it should be pointed out that the allosteric back-pocket DFG-out inhibitors will score as ATP-competitive.

Type-3 inhibitors that are further away from the ATP site are, for example, the ABL myristate-pocket (Myr-pocket) binders (Adrian *et al.*, 2006; Zhang *et al.*, 2009; Fabbro *et al.*, 2010), the CHEK1 inhibitors occupying part of the substrate binding site (Converso *et al.*, 2009) and the Jun kinase-1 (JNK1) inhibitors, which bind in part to the MAPK insert region and A-loop (Comess *et al.*, 2011) or to the DEF (docking site for ERK) domain (Tzarum *et al.*, 2013) to only

cite a few (Figure 6). A more comprehensive review on the type-3 inhibitors has been recently assembled by Cowan-Jacob *et al.* (2014).

Rapamycin and its derivatives (rapalogs), which target specifically mTOR kinase in the context of the mTORC1 complex, appear to be further removed from the kinase domain as they seem to act in the context of the mTORC1 complex (Wang and Sun, 2009; Yang *et al.*, 2013). Targeting the extracellular domains of the receptor TPKs and others by peptide-mimetics, 'peptoids' or antibodies is another special case of type-3 inhibitors (Fleishman *et al.*, 2002; Udugamasooriya *et al.*, 2008; Cazorla *et al.*, 2010; Jura *et al.*, 2011; Christopoulos *et al.*, 2014). The extracellular domains of RTPKs can be targeted by monoclonal antibodies trastuzumab (Herceptin, Roche, Basel, Switzerland) and pertuzumab (Perjeta, Roche, Basel, Switzerland), which act at different domains with trastuzumab binding to domain IV and pertuzumab to subdomain II of the extracellular segments of the HER2 (neu) receptor respectively (Cho *et al.*, 2003; Hynes and Lane, 2005; Hsieh and Moasser, 2007). In contrast, small molecules such as SSR128129E, which target the extracellular D2D3 domains of the fibroblast growth factor receptor (FGFR), modulate signalling of the FGFR-RTKs (Bono *et al.*, 2013; Herbert *et al.*, 2013). Examples of approved type-3 inhibitors are trametinib and the rapamycins (Table 1). There are many potential topographically distinct binding sites on kinases between which allosteric interactions can occur; the point from which the interaction is viewed drives classification of interacting ligands.

Summary of binding modes. While it is undisputed that type-3 inhibitors display the highest degree of selectivity, the implication that type-2 inhibitors are generally more selective than type-1 lacks quantitative arguments. In fact, there are examples of exquisitely selective inhibitors known for both types. Selectivity rests on particular features of a particular protein kinase, regardless of the binding mode of the inhibitor. In contrast to type-1 and type-2 inhibitors, the high level of selectivity of type-3 inhibitors reflects the unique binding sites and is off-set by the difficulty in obtaining and optimizing chemical matter (Converso *et al.*, 2009; Vanderpool *et al.*, 2009). So far, there are only sparse hints that allosteric sites can be generalized and chemical matter transferred to other kinases (Tecle *et al.*, 2009). There is currently no general strategy for the identification of allosteric kinase inhibitors or activators as most of them have been discovered serendipitously by diverse approaches ranging from phenotypic screening to sophisticated structure-based drug design.

Activators, paradoxical activation and priming

In addition, targeting allosteric sites on protein kinases may provide a means also to identify activators rather than inhibitors, which could be useful for therapeutic intervention as is the case for the glucokinase and the AMP-dependent protein kinase (AMPK) (Guertin and Grimsby, 2006; Sanders *et al.*, 2007). This can be useful for therapeutic intervention or as a pharmacological tool to better understand the biology of the protein or lipid kinase.

In particular, compounds targeting the PIF (PDK1 interacting fragment) pocket (the hydrophobic motif present in the N-terminal lobe of the AGC kinases) of either PDK1 (3-phosphoinositide-dependent protein kinase-1) or PKC ζ can either act as activators (Hindie *et al.*, 2009) or as substrate selective inhibitors (Figure 6) (Lopez-Garcia *et al.*, 2011; Sadowsky *et al.*, 2011; Busschots *et al.*, 2012). Similarly, the Myr-pocket binders of ABL can be converted into activators if they are designed not to allow bending of the I-helix of the ABL kinase domain (Jahnke *et al.*, 2010; Yang *et al.*, 2011).

However, there are a few protein kinases that require activation rather than inhibition to fulfil their therapeutic need, like the AMPK or the insulin receptor for which activators have been identified (Li *et al.*, 2001; Pender *et al.*, 2002; Sanders *et al.*, 2007; Lee *et al.*, 2011; Salt and Palmer, 2012; Hardie, 2014). PKC activation by exogenous compounds can have tumour-promoting or tumour-suppressing effects by acting via the DAG binding site (Martiny-Baron and Fabbro, 2007). These include phorbol esters, bryostatin and other compounds acting as DAG mimetic (Martiny-Baron and Fabbro, 2007). Other examples of kinase activators include a mimetic of the brain-derived neurotrophic factor that activates TrkB [tropomyosin receptor kinase B or neurotrophin receptor kinase-2 (NTRK-2)] (Massa *et al.*, 2010).

In some cases, kinase inhibitors can lead to unintended paradoxical activation either directly or via modulation of feedback loops. Evolution has endowed the signalling cascades of kinases with a high degree of robustness, which is achieved through redundancy at various levels, like compensatory pathways or protein expression, counteracting phosphatases and feedback loops. Therapeutic inhibition of kinases struggles with this innate inertia of kinase signalling. The most striking example is the paradoxical activation of selective BRAF inhibitors, which can activate the MAPK pathway in certain genetic backgrounds (Hall-Jackson *et al.*, 1999). This phenomenon is linked to a complex regulation of BRAF and CRAF due to cross-activation of the wild-type (wt) rapidly accelerated fibrosarcoma (RAF) isoforms, which is just beginning to be understood, almost a decade after the first so-called RAF inhibitor sorafenib was approved (Hall-Jackson *et al.*, 1999; Hatzivassiliou *et al.*, 2010; Poulikakos *et al.*, 2010; Holderfield *et al.*, 2013).

Another phenomenon is priming, which can lead to activation via kinase inhibitors and which has been observed for several kinases such as Akt, MEK and JAK (Okuzumi *et al.*, 2009; Andraos *et al.*, 2012; Hatzivassiliou *et al.*, 2013; Holderfield *et al.*, 2013). Priming describes the up-regulation of the phosphorylated form of the targeted kinase upon inhibition, which can lead to the activation of the pathway once the inhibitor is removed. Priming depends upon the mode of action of the kinase inhibitor. Inhibitors binding to the active conformation of Akt cause priming, whereas allosteric inhibitors targeting the inactive conformation of Akt do not (Lin *et al.*, 2012). This distinction was shown to depend upon the accessibility of the complex to its phosphatase PP2A (protein Ser/Thr-specific phosphatase-2). A broader understanding of priming and its impact on the efficacy of kinase inhibition is in its infancy, but there are reports that it could contribute to the lack of efficacy for certain inhibitors.

Another way by which kinase inhibitors influence the target kinase is by stabilizing and increasing its expression. In

the case of lapatinib, this leads to the expected inhibition of HER2, which at the same time resulted in an accumulation of HER2 due to decreased degradation, which leads to enhanced trastuzumab-dependent cytotoxicity (Scaltriti *et al.*, 2009). This is mainly due to the inhibition of dimerization, which is not common to all EGFR inhibitors (Sanchez-Martin and Pandiella, 2012).

Methods for discovering and profiling kinase inhibitors

In vitro biochemical and cellular assays followed by *in vivo* efficacy are the traditional pillars for drug discovery approaches (Knight *et al.*, 2013). The currently available technologies for the discovery and profiling of kinase-based drugs are numerous and it should be emphasized that the assessment of biochemical kinome-wide selectivity has only become available recently. There are a variety of biochemical protein kinase assays, including detection of radiolabelled transfer of phosphate to the substrate, ATP consumption or ADP production measurement, time-resolved FRET, peptide array-based, microfluidic technologies and label-free analysis (biophysical methods such as isothermal titration calorimetry and differential scanning fluorimetry) (Jia *et al.*, 2008; Ma *et al.*, 2008). Among the various providers that offer a kinase selectivity panel are KinomeScan™ of DiscoverX (<http://www.discoverx.com/targets/kinase-target-biology>), Millipore's Kinase Profiler (http://157.93.252.5/life_sciences/flx4/ld_kinaseprofiler_service) and Reaction Biology (<http://www.reactionbiology.com/webapps/site/>), which, to date, have the broadest kinome coverage (Karaman *et al.*, 2008; Anastasiadis *et al.*, 2011; Davis *et al.*, 2011; Gao *et al.*, 2013). For the assessment of biochemical selectivity, of course, the various formats of the various assays may change the overall selectivity. Although the biochemical assays of the above three providers are quite different, the IC₅₀ of a series of kinase inhibitors have been shown to be in reasonable agreement. Besides the caveats regarding assay formats, correlation to cellular selectivity, activation state of the recombinant kinase and the usually poor physicochemical properties of most inhibitors requires cautious interpretation of results. Ideally, the biochemical selectivity should match the cellular selectivity, which, to date, cannot be achieved with the same coverage as with the biochemical profile (Knight and Shokat, 2005; Knight *et al.*, 2013). Similar to the biochemical assay, the cellular assays for screening and profiling of kinase inhibitors come in different formats. Target profiler assays detect kinase proximal substrates by methods such as Western blots, phospho-ELISAs, reverse phase arrays, ALPHA (amplified luminescent proximity homogeneous assay)-screen assay and high content cellular analysis, and are being offered by various providers (Chen *et al.*, 2005; Warmuth *et al.*, 2007; Eglen *et al.*, 2008; Jia *et al.*, 2008). In addition, engineered cellular assays, such as BaF3, reporter gene assays, cell encyclopaedias and others, are particularly suited to obtain an integrated readout of the signalling cascade (Melnick *et al.*, 2006; Warmuth *et al.*, 2007; Barretina *et al.*, 2012). Cellular assay with a high kinome coverage may use biotinylated acyl phosphates of ATP and ADP that irreversibly react with

protein kinases on the conserved active site lysine residues in the ATP binding pocket followed by quantitative mass spectrometry (<http://www.kinativ.com/technology.html>) (Patricelli *et al.*, 2007; 2011). Alternatively, chemical proteomics can also probe the effectiveness of kinase inhibitors in cells and tissues (Bantscheff *et al.*, 2007). Finally, the function of the inhibition of the kinase target is being studied in specific tailor-made cell-based assays.

Taken together, the biochemical, cell-based phosphorylation and functional assays, most likely multiplexed, deliver a direct readout on the kinase activity in a cellular context and allow an *in vitro* activity of kinase inhibitors revealing on-target and off-target effects. Thus, the systematic profiling of inhibitors in broad arrays of biochemical and cellular assays has provided novel ways to better define the selectivity profile of drug candidates, including the potential for the discovery of novel mechanisms of actions. The most important contribution of profiling of compounds in large kinase panels is probably the cross-fertilization between protein kinase projects.

Major issues in kinase drug discovery

Kinase inhibitors are the prototypes of the targeted therapy and are therefore plagued by the fact that they are, like all targeted therapies, tailor-made to a particular patient population with the particular abnormal molecular or cellular defect. Targeted therapies are the cornerstone of precision medicine, which has improved the diagnostic, stratification and targeted treatment of patients as well as to better predict the outcome of the disease treatment (<http://cancergenome.nih.gov/>, <https://www.broadinstitute.org/>, <https://www.sanger.ac.uk/>) (Sellers, 2011; Garay and Gray, 2012; Plenge *et al.*, 2013). Thus, successful targeting is ultimately assessed by producing selective pharmacological responses, which reduce or eliminate side effects that are not mechanism-related. The idea that molecular information improves the precision with which patients are categorized and treated has led to a fragmentation of the patient population most likely to respond to the target agents (Figure 7B). In contrast, the duration of responses to certain targeted therapies has been shown to be limited, resulting in a poor benefit for the treated patients (Engelman and Settleman, 2008a; Engelman, 2009; Corcoran *et al.*, 2011; Chong and Janne, 2013). Therefore, correcting one molecular or cellular target by targeted therapies may be effective in diseases that strongly depend upon this one target. The reality of advanced cancers or other diseases are that they have multiple molecular abnormalities resulting in the potential for short-term efficacy (limited clinical benefit), which are usually associated with high costs (Pao and Hutchinson, 2012; Kantarjian *et al.*, 2013) (Figure 7B). In addition, in many cases, targeted therapies require chronic treatment and therefore the dependency on drugs to maintain molecular and cellular changes for the balance of life (Druker *et al.*, 2006).

The degree of selectivity a protein kinase inhibitor should ideally have has been and remains a controversial issue. The ideal kinase inhibitor should inhibit only the target kinase, which is usually almost impossible to achieve in the face of the over 500 protein kinases of the human kinome. Never-

theless, protein kinase inhibitors with a lower degree of selectivity have been hailed as ideal for oncological indications due to their potential for poly-pharmacology (Figure 7C) (Knight and Shokat, 2005; Force *et al.*, 2007; Morphy and Rankovic, 2007; Goldstein *et al.*, 2008; Karaman *et al.*, 2008; Anastassiadis *et al.*, 2011; Davis *et al.*, 2011; Gao *et al.*, 2013). A look at clinically advanced kinase inhibitors reminds us that a promiscuous selectivity profile, such as that of sunitinib, may be tolerated in oncological settings, albeit with some side effects (Figure 7C) (<http://www.discoverx.com/tools-resources/interaction-maps>). However, even some of the most selective inhibitors, particularly the allosteric inhibitors for mTOR or MEK1, can have serious dose-limiting on-target toxicity (Chhajed *et al.*, 2006; Akinleye *et al.*, 2013). With the exception of the highly selective lapatinib, tofacitinib, the rapalogs and trametinib, most of the other ATP-site-directed protein kinase inhibitors marketed derive their efficacy, at least in part, from their poly-pharmacology (<http://www.discoverx.com/tools-resources/interaction-maps>) (Figure 7C). In any case, for pharmacological target validation as well as chronic administration of kinase inhibitors in non-oncological indications, a reasonable selectivity is a prerequisite (Knight and Shokat, 2005; Goldstein *et al.*, 2008; Karaman *et al.*, 2008; Anastassiadis *et al.*, 2011; Davis *et al.*, 2011; Gao *et al.*, 2013).

Despite several successes over the past few years with kinase inhibitors, in most cases, the dependence of a disease state on the target kinase is either not known, poorly understood or displays a high degree of complexity, particularly in cancer. This often makes the selection of patients most likely to respond to a given kinase inhibitor treatment an almost impossible task (Fabbro *et al.*, 2012). Ongoing efforts using genome-wide screening, analysis of driver mutations in conjunction with the use of sophisticated disease models will unravel new disease associations and will pave the way for the discovery of many more new protein kinase targets in the coming years (Sellers, 2011).

In addition, understanding and predicting the cross-reactivity of kinase inhibitors in conjunction with the knowledge about the disease dependency of the target kinase would allow a more rapid proof of concept in the clinic. As discussed before, the selectivity of kinase inhibitors remains controversial. Unfortunately, we still poorly understand the selectivity profile with respect to their liabilities regarding preclinical toxicity findings and their relevance in patients (Yang *et al.*, 2010). The recent progress made in molecular profiling in conjunction with precision medicine will further our understanding towards a better assessment and prediction of efficacy/toxicity of these inhibitors in disease models [pharmacokinetic/pharmacodynamic (PK/PD)] and patients (Gray-Schopfer *et al.*, 2007; Zhang *et al.*, 2009; Courtney *et al.*, 2010; Fabbro *et al.*, 2012; London, 2013).

While we can expect more approvals for kinase inhibitors to come, the challenges of finding selective compounds with good physicochemical and PK properties remain and the intellectual property space is crowded.

Despite their central role in biology and their sizable potential as therapeutic targets, only a small fraction of the human protein kinases have been functionally annotated. In addition, we are short of selective small molecule kinase inhibitors to address unmet medical need in cancer,

A • Targeted therapies

- Small patient populations (fragmentation)
- Clinical benefit ?
- Biomarkers for diagnosis, prediction, stratification and disease outcome

• Kinase targeted therapies

- On/off target pharmacology (selectivity)
- 500 + kinase genes (< 20% of the kinome explored as target)
- New modes of inhibition & Novel scaffolds
- Drug resistance (mainly in Oncology)
 - Mutations in target kinase(s)
 - Pathway reactivation & bypass mechanisms
 - Pathway independent (μ env, EMT etc.)
- Only a limited number kinase inhibitors in non-oncological indications

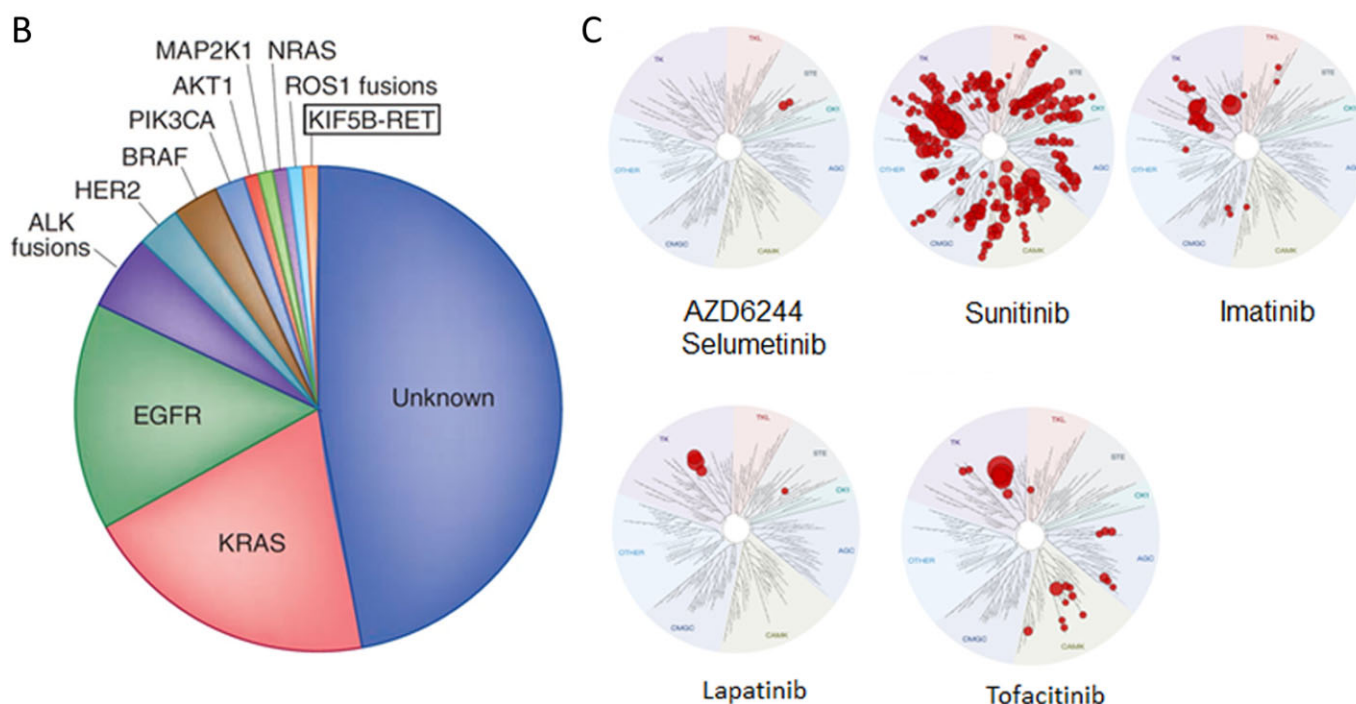


Figure 7

(A) Major issues in kinase drug discovery. (B) Pie chart showing the percentage distribution of clinically relevant driver mutations in lung adenocarcinoma [adapted from with high costs 'Chipping away at the lung cancer genome' by Pao and Hutchinson (2012)]. (C) Selectivity of selected approved protein kinase inhibitors as determined by the DiscoverX KinomeScan. The human kinome is represented as circular phylogenetic tree without the atypical protein kinases and results are reported as a map (Treespot), which allows visualizing compound interactions across the human kinome panel. AZD6244 (selumetinib) is an allosteric MEK inhibitor which displays the same selectivity as trametinib. Data are taken from <http://www.discoverx.com/tools-resources/interaction-maps>.

metabolism, inflammation and other diseases (Fedorov *et al.*, 2010; Knapp *et al.*, 2013). On the contrary, there are many 'specific' protein kinase inhibitors that cannot be used as drugs for reasons of toxicity or solubility but which are extremely useful as research reagents to better understand the cellular networking in normal and diseased tissues (Robert *et al.*, 2005; Force *et al.*, 2007). Using low-molecular-weight compounds offers considerable advantages in experimental demands and interpretation of results over RNA interference

techniques and genetic knockout or knock-in models, which are limited by the kinetics of their effects and the inability to discriminate between scaffolding and catalytic roles of the target protein. Thus, selective chemical probes to functionally annotate, in particular, the untapped kinome could stimulate new drug discovery efforts to address unmet medical needs. Since the size of the human kinome combined with the high cost associated with probe generation severely limits access to new probes, potentially a large-scale

public-private partnership may minimize redundancy and sharing of risk and cost (Knapp *et al.*, 2013).

The major challenge for kinase drug discovery is not only to better understand the disease dependence of the target kinase but also to anticipate the emerging resistance to kinase inhibitors under treatment. Kinase inhibitors are being and have been designed to specifically target kinase alleles with GOFs (Blume-Jensen and Hunter, 2001; Fabbro and Garcia-Echeverria, 2002a). Despite these successes, it should be emphasized that patients most likely to benefit from these kinase inhibitors often relapse after an initial response. Thus, emergence of drug resistance is not limited to conventional chemotherapeutic drugs but extends to drugs with a targeted mode of action (Engelman and Settleman, 2008a).

Resistance to kinase inhibition

The mechanisms of multidrug resistance (MDR) to chemotherapeutic drugs have been studied and are not only limited to reduced drug accumulation but also involve changes in the level of target proteins, mutations which diminish drug binding, trapping of drugs in acidic vesicles, enhanced metabolism of drugs by cytochrome P450 (CYP) mixed function oxidases, increased tolerance of cellular DNA damage and diminished apoptotic signalling (Gottesman, 2002; Szakacs *et al.*, 2006; Hall *et al.*, 2009). Apart from the usual mechanisms of drug inactivation in cancer as well as the findings that quiescent tumour stem cells are refractory to kinase inhibitors (Graham *et al.*, 2002), there are additional target-related mechanisms for resistance that are not based upon mutations of the target kinase. Drug resistance to targeted agents such as kinase inhibitors can occur either by compensatory mechanisms or by reducing the affinity of the kinase to its inhibitors (Szakacs *et al.*, 2006; Fabbro *et al.*, 2011).

In its simplest way, protein kinases escape inhibition by mutating key residues in their catalytic domains (Hunter, 2000; Gorre *et al.*, 2001; Kobayashi *et al.*, 2005; Takano *et al.*, 2005; Ventura and Nebreda, 2006; Ali and Ali, 2007; Engelman *et al.*, 2007; Chandarlapaty *et al.*, 2011). The most commonly found point mutation leading to resistance concomitant with relapses affects the gatekeeper residue whose size and shape regulate the properties of the hydrophobic pocket located at the back of the ATP binding site. These mutations include the Thr-gatekeeper of BCR-ABL1 (T315I) (Gorre *et al.*, 2001; Sawyers, 2004; Fabbro *et al.*, 2005), KIT (T670I) (Heinrich *et al.*, 2003; Fletcher and Rubin, 2007), platelet-derived growth factor receptor- α (PDGFR α) (T674I) (Cools *et al.*, 2003), PDGFR β (T681I) (Daub *et al.*, 2004) and Src (proto-oncogene tyrosine-protein kinase Src) T341M (Bishop, 2004), as well as other types of gatekeepers such as L1196M in ALK (Katayama *et al.*, 2012), G697R in FLT3 (fetal liver kinase-3) (Cools *et al.*, 2004) and V561M in FGFR1 (fibroblast growth factor receptor-1) (Blencke *et al.*, 2004). Loss of affinity to the kinase inhibitor is either due to a steric clash between inhibitor and the mutated gatekeeper, like in the case of BCR-ABL, or by significantly increasing the affinity for ATP and thereby reducing the affinity for the kinase inhibitors, like in the case of the EGFR (Daub *et al.*, 2004; Kobayashi *et al.*, 2005; Pao *et al.*, 2005). Inhibitors targeting the inactive

conformation of protein kinases are generally more prone to resistance mutations. In this case, a single mutation can act both by destroying crucial interactions and/or destabilizing the target conformation. Two frequent types of activating mutation in the gatekeeper and in the A-loop serve to illustrate the link between mutation and conformation. While the gatekeeper mutation is well conserved (Azam *et al.*, 2008), the A-loop mutations are diverse (Dibb *et al.*, 2004). Both types of mutations may activate the kinase (Azam *et al.*, 2008). In addition to blocking access to the hydrophobic back-pocket (which is detrimental to ligand binding), mutation of a small to a large hydrophobic gatekeeper also stabilizes the active conformation, presumably by stabilization of the R-spine (Kornev *et al.*, 2006; Taylor and Kornev, 2011).

In addition, mutations in MEK1 and ABL illustrate the diversity of the resistance mechanisms: (i) although many different mutations have been reported for MEK1 also in the context of the RAF resistance (Van Allen *et al.*, 2014), they seem to work by a common mechanism in which the interface with the autoinhibitory N-terminal helix is disrupted or (ii) in the case of ABL, the most common and most resistant mutations interfere directly or indirectly with the binding of the drug, but other mutations map all over the kinase domain and it is unclear how some of these confer resistance (Apperley, 2007). The most dominant mutation in terms of resistance in both ABL and ALK is due to the gatekeeper mutations T315I and L1196M, which make them insensitive to imatinib and crizotinib respectively (Figure 5).

Mutations of the gatekeeper as well as other kinase domain mutations confer resistance to a wide spectrum of kinase inhibitors without affecting the kinase activity and may explain a fraction of cases of acquired resistance. The resistance mechanisms to kinase inhibitors are multiple and aim, in cancer, in the large part to restore the activity of the original 'cancer-addicting' pathway. This can occur either by conformational changes in the kinase domain or by reactivating the pathway downstream and/or parallel to the targeted kinase (Hunter, 2000; Gorre *et al.*, 2001; Sawyers, 2004; Kobayashi *et al.*, 2005; Takano *et al.*, 2005; Rubin and Duensing, 2006; Ventura and Nebreda, 2006; Ali and Ali, 2007; Engelman *et al.*, 2007; Chandarlapaty *et al.*, 2011; Fabbro *et al.*, 2011; Serra *et al.*, 2011; Logue and Morrison, 2012; Trusolino and Bertotti, 2012; Workman *et al.*, 2013b). Compensatory changes in the signalling pathways bypassing the drug-mediated inhibition and restoring the inhibited signalling pathway include the following:

- 1 amplification of the target kinase like BCR-ABL in CML (le Coutre *et al.*, 2000) or dimerization of aberrantly spliced BRAF(V-600E) (Poulikakos *et al.*, 2011)
- 2 up-regulation of receptor TPKs following either inhibition of PI3K (Serra *et al.*, 2011; Rodon *et al.*, 2013) or up-regulation of MET, IGF1R or AXL (AXL tyrosine kinase) in the acquisition of resistance to EGFR kinase inhibition (Engelman *et al.*, 2007; Turke *et al.*, 2010; Logue and Morrison, 2012)
- 3 activation of the RAS-RAF-MAPK and/or PI3K/Akt pathways by several mechanisms can override the effects of receptor TPK inhibitors by activating point mutations in PI3K, LOF/deletions of the PTEN (phosphatase and tensin

homologue) phosphatase, activation of RAS isoforms, activation of COT (cancer Osaka thyroid aka MAP3K8 aka Tpl2; the immunological counterpart of RAF) (She *et al.*, 2003; Johannessen *et al.*, 2010; Corcoran *et al.*, 2011; Prahallad *et al.*, 2012)

4 signalling redundancies, interconnections through pathway crosstalk and feedback loops have also been identified as contributors to drug resistance (Janne *et al.*, 2009; O'Reilly and McSheehy, 2010; Mendoza *et al.*, 2011; Rodrik-Outmezguine *et al.*, 2011; Chandarlapaty, 2012; Logue and Morrison, 2012; Trusolino and Bertotti, 2012). Allosteric inhibition of mTORC1 by rapamycins leads to disruption of a negative feedback loop, which activates Akt counteracting its anti-proliferative effects (Chandarlapaty, 2012). Inhibition of PI3K/mTOR signalling may lead to activation of the JAK/STAT5 pathway (Britschgi *et al.*, 2012), while inhibition of mutant V600E-BRAF by vemurafenib in cells with oncogenic RAS causes unexpected activation of the MAPK cascade by favouring the formation of wt BRAF and CRAF dimerization which can result in kerato-acanthomas in patients (Chapman *et al.*, 2011; Poulikakos *et al.*, 2011).

5 Factors regulating the bioavailability and intracellular concentration of inhibitors, such as poor intestinal absorption, tight binding to blood plasma proteins, overexpression of the MDR genes and/or increased metabolism of the drug by liver cytochrome P450 proteins, have also been linked to primary resistance (Mahon *et al.*, 2003; Apperley, 2007).

All of these mechanisms demonstrate the plasticity of cancer cells and the many ways by which a tumour can evade targeted therapies. Strategies have been deployed to override these various types of resistances, including compounds capable of circumventing the target-related drug resistance by developing 'second-generation' kinase inhibitors (Lombardo *et al.*, 2004; Weisberg *et al.*, 2005; Adrian *et al.*, 2006; Quintas-Cardama *et al.*, 2007; Engelman *et al.*, 2008b; Fabbro *et al.*, 2010; Zhang *et al.*, 2010).

For example, inhibitors that bind covalently to the ATP binding site of EGFR have been developed for the emerging resistance to gefitinib and erlotinib (Kwak *et al.*, 2005; Heymach *et al.*, 2006; Felip *et al.*, 2007; Zhou *et al.*, 2011). Several of these covalent inhibitors are in late stage clinical trials (Zhang *et al.*, 2009; Liu *et al.*, 2013). Alternatively, these type of covalent inhibitors, as in the case of ibrutinib, have been designed upfront to bind covalently to Cys⁴⁸¹ of BTK and recently approved for B-cell malignancies (Byrd *et al.*, 2013; Wiestner, 2013; Akinleye *et al.*, 2014). Although ibrutinib has shown impressive clinical results, patients that have disease progression revealed a C481S mutation in their BTK that abrogates the covalent binding to ibrutinib (Furman *et al.*, 2014).

Non-covalent inhibitors that can tolerate the amino acid exchange at the gatekeeper position have also been developed and, like ponatinib, approved for the T315I ABL gatekeeper mutant (O'Hare *et al.*, 2009; Hoy, 2014). Targeting the gatekeeper mutation usually leads to low selectivity with deleterious side effects, leading to retraction from the market due to safety issues (Force *et al.*, 2007; Cheng and Force, 2010; Dalzell, 2013).

A further approach is to target the kinase outside the ATP binding sites with the goal of combining the ATP-site-directed inhibitors (type-1 and type-2) with the type-3 inhibitors (Cowan-Jacob *et al.*, 2014). A remote binding site on the kinase domain is addressed by the GNF-2 compound, which was found by a phenotypic screen shown to target the Myr-pocket binding site of ABL (Adrian *et al.*, 2006; Fabbro *et al.*, 2010; Zhang *et al.*, 2010). Exploration of the combined efficacy between the Myr-pocket and ATP binding sites significantly increased the survival of mice in bone marrow transplantation CML models compared to treatment with either agent alone (Zhang *et al.*, 2010). In addition, the improved potency of second-generation Myr-pocket binder against wt ABL and T315-ABL also translated into a high level of degree of synergy in BaF3 cells transformed with BCR-ABL-T315I when combined with ATP-site-directed inhibitors such as nilotinib or dasatinib, as has been noted in previous studies (Fabbro *et al.*, 2002b; Zhang *et al.*, 2010). Surprisingly, NMR and small angle X-ray scattering analyses revealed an open state of the ABL when bound to ATP-site-directed inhibitors, such as imatinib, leading to the detachment of the SH3-SH2 domains from the kinase domain and the formation of an 'open' inactive state, which is inhibited in the ATP site, which can be reversed by the addition of the Myr-pocket binder (Skora *et al.*, 2013). Whether these data explain the synergy between the Myr-pocket binder and ATP-directed inhibitors which appear to overcome the T315I-ABL-mediated drug resistance remains to be seen. The findings on the actions of the two classes of inhibitors on a single target kinase may help to devise new strategies for drug development.

Another approach is to combine different kinase inhibitors targeting kinases of the same pathway like in the case of vemurafenib where the emerging resistance is not due to mutations in the B-Raf (V600E) but rather in the downstream MEK1 (Wagle *et al.*, 2011; Medina *et al.*, 2013). This has recently resulted in the approval of the dabrafenib (a RAF inhibitor) and trametinib (a MEK1 inhibitor) combination for metastatic melanoma (Table 1) (King *et al.*, 2013).

Unfortunately, only a very limited number of non-ATP-competitive kinase inhibitors have thus far been identified, which could also be used to address the resistance caused by mutations in the ATP binding site (Cowan-Jacob *et al.*, 2014). In addition, predicting clinical resistance to the targeted kinase inhibitor therapy is a gamble. Taking the example of BCR-ABL, saturation mutagenesis could predict most of the imatinib-resistant mutants in the kinase domain found in the clinic (Azam *et al.*, 2003; 2008). In contrast, adopting a similar approach as the B-Raf (V600E) or MEK1 would have failed as the acquired resistance is multiple and mainly due to reactivation of the signalling pathway (Corcoran *et al.*, 2011). It should be emphasized that detecting clinical resistance is difficult to resolve due to the paucity of matched biopsies and limited coverage even of next-generation sequencing panels.

Resistance to kinase inhibitors in non-oncological indications is less likely to occur, as the selection pressure for the disease causing cell survival and the complexity of the destabilized genome, as is often the case in advanced cancers, is lower.

Thus, resistance to protein kinase inhibitors can emerge in several ways under treatment, raising the issue of an

endless chase of resistance alleles, with ever more specific inhibitors.

A comprehensive combination of inhibitors, which take care of the resistance in the target kinase as well as of compensatory signalling, will be required to combat the emerging resistance in targeted cancer therapies. The only way to approach these problems is to use a rational combination of drugs.

Future perspectives in kinase drug discovery

Kinase inhibitor drug discovery has evolved into a mature field, with a wealth of structural and biological insights, as well as pharmacological tools. At the same time, we have only scratched the surface of the target space and are continuously humbled by the complexity of signalling pathways. The field has seen a lot of reasons why a target can fail regardless of how compelling the genetics may be. We often discover unexpected biology upon pharmacological inhibition. We have yet to understand feedback, compensatory mechanisms and resistance mechanisms better. We have yet to understand target toxicity and how it translates from preclinical species to man. State-of-the-art kinase drug discovery needs to take into account all of these subtleties and incorporate the lessons learned to succeed with the kinase targets of the future.

Understanding the conformational changes of protein kinases, which as molecular switches transition from the 'on-' and 'off-states', will allow for a better design of inhibitors and will provide a common framework for understanding the activation of the kinase, disease causality, therapeutic modalities and resistance. Aberrant activation of protein kinases occurs by pushing the equilibrium towards a constitutive active conformation, which is very similar in all protein kinases and in its essence can be defined by the DFG-motif forming the typical turn-hairpin-turn conformation in tight contact with the C-helix.

The large number of kinase inhibitors in clinical development will ensure a constant flow of novel targeted therapies, with increasing numbers in non-oncological indications, to the clinic over the next 10 years. The vast majority of kinase inhibitors are, at present, for various oncology indications, which not only reflects the more acute nature of the disease but also the greater tolerability with respect to potential side effects. The future of protein kinase-targeted therapeutics in cancer appears promising, despite the fact that several protein kinase inhibitors that have entered human clinical trials are not very specific and did not achieve the anticipated results. This situation may be improved by the upcoming second generation of kinase inhibitors with a better selectivity that will be applied to a genetically better defined patient population. The development of kinase inhibitors for non-life-threatening indications where chronic regimens are being used will require *a priori* a better target selectivity to minimize side effects. Identification of highly selective kinase inhibitors and activators should lead to an expansion of the chemical and biological kinase space, as well as to an improved understanding of their therapeutic limitations and potentials.

Acknowledgements

NC-IUPHAR receives financial support from the Wellcome Trust.

References

- Adrian FJ, Ding Q, Sim T, Velentza A, Sloan C, Liu Y *et al.* (2006). Allosteric inhibitors of Bcr-abl-dependent cell proliferation. *Nat Chem Biol* 2: 95–102.
- Akinleye A, Furqan M, Mukhi N, Ravella P, Liu D (2013). MEK and the inhibitors: from bench to bedside. *J Hematol Oncol* 6: 27.
- Akinleye A, Furqan M, Adekunle O (2014). Ibrutinib and indolent B-cell lymphomas. *Clin Lymphoma Myeloma Leuk* 14: 253–260.
- Albrecht BK, Harmange JC, Bauer D, Berry L, Bode C, Boezio AA *et al.* (2008). Discovery and optimization of triazolopyridazines as potent and selective inhibitors of the c-Met kinase. *J Med Chem* 51: 2879–2882.
- Alexander SPH, Benson HE, Faccenda E, Pawson AJ, Sharman JL, Spedding M *et al.* (2013a). The Concise Guide to PHARMACOLOGY 2013/14: Catalytic receptors. *Br J Pharmacol* 170: 1676–1705.
- Alexander SPH, Benson HE, Faccenda E, Pawson AJ, Sharman JL, Spedding M *et al.* (2013b). The Concise Guide to PHARMACOLOGY 2013/14: Enzymes. *Br J Pharmacol* 170: 1797–1867.
- Ali S, Ali S (2007). Role of c-kit/SCF in cause and treatment of gastrointestinal stromal tumors (GIST). *Gene* 401: 38–45.
- Amsberg GK, Koschmieder S (2013). Profile of bosutinib and its clinical potential in the treatment of chronic myeloid leukemia. *Onco Targets Ther* 6: 99–106.
- Anastassiadis T, Deacon SW, Devarajan K, Ma H, Peterson JR (2011). Comprehensive assay of kinase catalytic activity reveals features of kinase inhibitor selectivity. *Nat Biotechnol* 29: 1039–1045.
- Andraos R, Qian Z, Bonenfant D, Rubert J, Vangrevelinghe E, Scheufler C *et al.* (2012). Modulation of activation-loop phosphorylation by JAK inhibitors is binding mode dependent. *Cancer Discov* 2: 512–523.
- Angulo I, Vadas O, Garcon F, Banham-Hall E, Plagnol V, Leahy TR *et al.* (2013). Phosphoinositide 3-kinase delta gene mutation predisposes to respiratory infection and airway damage. *Science* 342: 866–871.
- Ansari J, Hussain SA, Ansari A, Glaholm J (2013). Critical appraisal of axitinib in the treatment of advanced renal cell carcinoma. *Biologics* 7: 39–46.
- Apperley JF (2007). Part I: mechanisms of resistance to imatinib in chronic myeloid leukaemia. *Lancet Oncol* 8: 1018–1029.
- Azam M, Raz T, Nardi V, Opitz SL, Daley GQ (2003). A screen to identify drug resistant variants to target-directed anti-cancer agents. *Biol Proced Online* 5: 204–210.
- Azam M, Seeliger MA, Gray NS, Kuriyan J, Daley GQ (2008). Activation of tyrosine kinases by mutation of the gatekeeper threonine. *Nat Struct Mol Biol* 15: 1109–1118.
- Ballantyne AD, Garnock-Jones KP (2013). Dabrafenib: first global approval. *Drugs* 73: 1367–1376.

- Bantscheff M, Eberhard D, Abraham Y, Bastuck S, Boesche M, Hobson S *et al.* (2007). Quantitative chemical proteomics reveals mechanisms of action of clinical ABL kinase inhibitors. *Nat Biotechnol* 25: 1035–1044.
- Bardelli A, Parsons DW, Silliman N, Ptak J, Szabo S, Saha S *et al.* (2003). Mutational analysis of the tyrosine kinome in colorectal cancers. *Science* 300: 949.
- Barker AJ, Gibson KH, Grundy W, Godfrey AA, Barlow JJ, Healy MP *et al.* (2001). Studies leading to the identification of ZD1839 (IRESSA): an orally active, selective epidermal growth factor receptor tyrosine kinase inhibitor targeted to the treatment of cancer. *Bioorg Med Chem Lett* 11: 1911–1914.
- Barnett SF, Defeo-Jones D, Fu S, Hancock PJ, Haskell KM, Jones RE *et al.* (2005). Identification and characterization of pleckstrin-homology-domain-dependent and isoenzyme-specific Akt inhibitors. *Biochem J* 385: 399–408.
- Barretina J, Caponigro G, Stransky N, Venkatesan K, Margolin AA, Kim S *et al.* (2012). The Cancer Cell Line Encyclopedia enables predictive modelling of anticancer drug sensitivity. *Nature* 483: 603–607.
- Bartholomeusz C, Gonzalez-Angulo AM (2012). Targeting the PI3K signaling pathway in cancer therapy. *Expert Opin Ther Targets* 16: 121–130.
- Baselga J, Campone M, Piccart M, Burris HA 3rd, Rugo HS, Sahmoud T *et al.* (2012). Everolimus in postmenopausal hormone-receptor-positive advanced breast cancer. *N Engl J Med* 366: 520–529.
- Beck JT, Hortobagyi GN, Campone M, Lebrun F, Deleu I, Rugo HS *et al.* (2014). Everolimus plus exemestane as first-line therapy in HR(+), HER2(-) advanced breast cancer in BOLERO-2. *Breast Cancer Res Treat* 143: 459–467.
- Bishop AC (2004). A hot spot for protein kinase inhibitor sensitivity. *Chem Biol* 11: 587–589.
- Blencke S, Zech B, Engkvist O, Greff Z, Orfi L, Horvath Z *et al.* (2004). Characterization of a conserved structural determinant controlling protein kinase sensitivity to selective inhibitors. *Chem Biol* 11: 691–701.
- Blume-Jensen P, Hunter T (2001). Oncogenic kinase signalling. *Nature* 411: 355–365.
- Bono F, De Smet F, Herbert C, De Bock K, Georgiadou M, Fons P *et al.* (2013). Inhibition of tumor angiogenesis and growth by a small-molecule multi-FGF receptor blocker with allosteric properties. *Cancer Cell* 23: 477–488.
- Bornancin F (2011). Ceramide kinase: the first decade. *Cell Signal* 23: 999–1008.
- Boudeau J, Miranda-Saavedra D, Barton GJ, Alessi DR (2006). Emerging roles of pseudokinases. *Trends Cell Biol* 16: 443–452.
- Britschgi A, Andraos R, Brinkhaus H, Klebba I, Romanet V, Muller U *et al.* (2012). JAK2/STAT5 inhibition circumvents resistance to PI3K/mTOR blockade: a rationale for cotargeting these pathways in metastatic breast cancer. *Cancer Cell* 22: 796–811.
- Brooks AJ, Dai W, O'Mara ML, Abankwa D, Chhabra Y, Pelekanos RA *et al.* (2014). Mechanism of activation of protein kinase JAK2 by the growth hormone receptor. *Science* 344: 1249783.
- Buchdunger E, Matter A, Druker BJ (2001). Bcr-Abl inhibition as a modality of CML therapeutics. *Biochim Biophys Acta* 1551: M11–M18.
- Busschots K, Lopez-Garcia LA, Lammi C, Stroba A, Zeuzem S, Piiper A *et al.* (2012). Substrate-selective inhibition of protein kinase PDK1 by small compounds that bind to the PIF-pocket allosteric docking site. *Chem Biol* 19: 1152–1163.
- Byrd JC, Furman RR, Coutre SE, Flinn IW, Burger JA, Blum KA *et al.* (2013). Targeting BTK with ibrutinib in relapsed chronic lymphocytic leukemia. *N Engl J Med* 369: 32–42.
- Carlomagno F, Santoro M (2004). Identification of RET kinase inhibitors as potential new treatment for sporadic and inherited thyroid cancer. *J Chemother* 16 (Suppl. 4): 49–51.
- Cazorla M, Jouvenceau A, Rose C, Guilloux JP, Pilon C, Dranovsky A *et al.* (2010). Cyclotraxin-B, the first highly potent and selective TrkB inhibitor, has anxiolytic properties in mice. *PLoS ONE* 5: e9777.
- Chan HY, Grossman AB, Bukowski RM (2010). Everolimus in the treatment of renal cell carcinoma and neuroendocrine tumors. *Adv Ther* 27: 495–511.
- Chandarlapaty S (2012). Negative feedback and adaptive resistance to the targeted therapy of cancer. *Cancer Discov* 2: 311–319.
- Chandarlapaty S, Sawai A, Scaltriti M, Rodrik-Outmezguine V, Grbovic-Huezo O, Serra V *et al.* (2011). Akt inhibition relieves feedback suppression of receptor tyrosine kinase expression and activity. *Cancer Cell* 19: 58–71.
- Chapman PB, Hauschild A, Robert C, Haanen JB, Ascierto P, Larkin J *et al.* (2011). Improved survival with vemurafenib in melanoma with BRAF V600E mutation. *N Engl J Med* 364: 2507–2516.
- Chau NG, Haddad RI (2013). Vandetanib for the treatment of medullary thyroid cancer. *Clin Cancer Res* 19: 524–529.
- Chen H, Kovar J, Sissons S, Cox K, Matter W, Chadwell F *et al.* (2005). A cell-based immunocytochemical assay for monitoring kinase signaling pathways and drug efficacy. *Anal Biochem* 338: 136–142.
- Cheng H, Force T (2010). Why do kinase inhibitors cause cardiotoxicity and what can be done about it? *Prog Cardiovasc Dis* 53: 114–120.
- Chhajed PN, Dickenmann M, Bubendorf L, Mayr M, Steiger J, Tamm M (2006). Patterns of pulmonary complications associated with sirolimus. *Respiration* 73: 367–374.
- Chico LK, Van Eldik LJ, Watterson DM (2009). Targeting protein kinases in central nervous system disorders. *Nat Rev Drug Discov* 8: 892–909.
- Cho HS, Mason K, Ramyar KX, Stanley AM, Gabelli SB, Denney DW Jr *et al.* (2003). Structure of the extracellular region of HER2 alone and in complex with the Herceptin Fab. *Nature* 421: 756–760.
- Chong CR, Janne PA (2013). The quest to overcome resistance to EGFR-targeted therapies in cancer. *Nat Med* 19: 1389–1400.
- Christopoulos A, Changeux JP, Catterall WA, Fabbro D, Burris TP, Cidlowski JA *et al.* (2014). International Union of Basic and Clinical Pharmacology. XC. Multisite pharmacology: recommendations for the nomenclature of receptor allosterism and allosteric ligands. *Pharmacol Rev* 66: 918–947.
- Cibrik D, Silva HT Jr, Vathsala A, Lackova E, Cornu-Artis C, Walker RG *et al.* (2013). Randomized trial of everolimus-facilitated calcineurin inhibitor minimization over 24 months in renal transplantation. *Transplantation* 95: 933–942.
- Cohen MS, Hadjivassiliou H, Taunton J (2007). A clickable inhibitor reveals context-dependent autoactivation of p90 RSK. *Nat Chem Biol* 3: 156–160.
- Cohen P (2001). The role of protein phosphorylation in human health and disease. The Sir Hans Krebs Medal Lecture. *Eur J Biochem* 268: 5001–5010.

- Cohen P (2002a). The origins of protein phosphorylation. *Nat Cell Biol* 4: E127–E130.
- Cohen P (2002b). Protein kinases – the major drug targets of the twenty-first century? *Nat Rev Drug Discov* 1: 309–315.
- Comess KM, Sun C, Abad-Zapatero C, Goedken ER, Gum RJ, Borhani DW *et al.* (2011). Discovery and characterization of non-ATP site inhibitors of the mitogen activated protein (MAP) kinases. *ACS Chem Biol* 6: 234–244.
- Converso A, Hartingh T, Garbaccio RM, Tasber E, Rickert K, Fraley ME *et al.* (2009). Development of thioquinazolinones, allosteric Chk1 kinase inhibitors. *Bioorg Med Chem Lett* 19: 1240–1244.
- Cools J, Stover EH, Boulton CL, Gotlib J, Legare RD, Amaral SM *et al.* (2003). PKC412 overcomes resistance to imatinib in a murine model of FIP1L1-PDGFR α -induced myeloproliferative disease. *Cancer Cell* 3: 459–469.
- Cools J, Mentens N, Furet P, Fabbro D, Clark JJ, Griffin JD *et al.* (2004). Prediction of resistance to small molecule FLT3 inhibitors: implications for molecularly targeted therapy of acute leukemia. *Cancer Res* 64: 6385–6389.
- Corcoran RB, Settleman J, Engelman JA (2011). Potential therapeutic strategies to overcome acquired resistance to BRAF or MEK inhibitors in BRAF mutant cancers. *Oncotarget* 2: 336–346.
- Courtney KD, Corcoran RB, Engelman JA (2010). The PI3K pathway as drug target in human cancer. *J Clin Oncol* 28: 1075–1083.
- le Coutre P, Tassi E, Varella-Garcia M, Barni R, Mologni L, Cabrita G *et al.* (2000). Induction of resistance to the Abelson inhibitor STI571 in human leukemic cells through gene amplification. *Blood* 95: 1758–1766.
- Cowan-Jacob SW (2006). Structural biology of protein tyrosine kinases. *Cell Mol Life Sci* 63: 2608–2625.
- Cowan-Jacob SW, Möbitz H, Fabbro D (2009). Structural biology contributions to tyrosine kinase drug discovery. *Curr Opin Cell Biol* 21: 280–287.
- Cowan-Jacob SW, Jahnke W, Knapp S (2014). Novel approaches for targeting kinases: allosteric inhibition, allosteric activation and pseudokinases. *Future Med Chem* 6: 541–561.
- Dalzell MD (2013). Ponatinib pulled off market over safety issues. *Manag Care* 22: 42–43.
- Daub H, Specht K, Ullrich A (2004). Strategies to overcome resistance to targeted protein kinase inhibitors. *Nat Rev Drug Discov* 3: 1001–1010.
- Davies SP, Reddy H, Caivano M, Cohen P (2000). Specificity and mechanism of action of some commonly used protein kinase inhibitors. *Biochem J* 351: 95–105.
- Davis MI, Hunt JP, Herrgard S, Ciceri P, Wodicka LM, Pallares G *et al.* (2011). Comprehensive analysis of kinase inhibitor selectivity. *Nat Biotechnol* 29: 1046–1051.
- Dibb NJ, Dilworth SM, Mol CD (2004). Switching on kinases: oncogenic activation of BRAF and the PDGFR family. *Nat Rev Cancer* 4: 718–727.
- Druker BJ, Tamura S, Buchdunger E, Ohno S, Segal GM, Fanning S *et al.* (1996). Effects of a selective inhibitor of the Abl tyrosine kinase on the growth of Bcr-Abl positive cells. *Nat Med* 2: 561–566.
- Druker BJ, Guilhot F, O'Brien SG, Gathmann I, Kantarjian H, Gattermann N *et al.* (2006). Five-year follow-up of patients receiving imatinib for chronic myeloid leukemia. *N Engl J Med* 355: 2408–2417.
- Eglen RM, Reisine T, Roby P, Rouleau N, Illy C, Bosse R *et al.* (2008). The use of AlphaScreen technology in HTS: current status. *Curr Chem Genomics* 1: 2–10.
- Engelman JA (2009). Targeting PI3K signalling in cancer: opportunities, challenges and limitations. *Nat Rev Cancer* 9: 550–562.
- Engelman JA, Settleman J (2008a). Acquired resistance to tyrosine kinase inhibitors during cancer therapy. *Curr Opin Genet Dev* 18: 73–79.
- Engelman JA, Zejnullahu K, Mitsudomi T, Song Y, Hyland C, Park JO *et al.* (2007). MET amplification leads to gefitinib resistance in lung cancer by activating ERBB3 signaling. *Science* 316: 1039–1043.
- Engelman JA, Chen L, Tan X, Crosby K, Guimaraes AR, Upadhyay R *et al.* (2008b). Effective use of PI3K and MEK inhibitors to treat mutant Kras G12D and PIK3CA H1047R murine lung cancers. *Nat Med* 14: 1351–1356.
- Fabbro D, Garcia-Echeverria C (2002a). Targeting protein kinases in cancer therapy. *Curr Opin Drug Discov Devel* 5: 701–712.
- Fabbro D, Ruetz S, Buchdunger E, Cowan-Jacob SW, Fendrich G, Liebetanz J *et al.* (2002b). Protein kinases as targets for anticancer agents: from inhibitors to useful drugs. *Pharmacol Ther* 93: 79–98.
- Fabbro D, Fendrich G, Guez V, Meyer T, Furet P, Mestan P *et al.* (2005). Targeted therapy with imatinib: an exception or a rule? *Handb Exp Pharmacol* 167: 361–389.
- Fabbro D, Manley PW, Jahnke W, Liebetanz J, Szyttenholm A, Fendrich G *et al.* (2010). Inhibitors of the Abl kinase directed at either the ATP- or myristate-binding site. *Biochim Biophys Acta* 1804: 454–462.
- Fabbro D, Cowan-Jacob SW, Möbitz H, Martiny-Baron G (2011). Targeting cancer with small-molecular-weight kinase inhibitors. *Methods Mol Biol* 795: 1–34.
- Fabbro D, Cowan SW, Möbitz H, Martiny-Baron G (2012). Targeting cancer with small-molecular-weight kinase inhibitors. *Methods Mol Biol* 795: 1–34.
- Faivre S, Demetri G, Sargent W, Raymond E (2007). Molecular basis for sunitinib efficacy and future clinical development. *Nat Rev Drug Discov* 6: 734–745.
- Fedorov O, Muller S, Knapp S (2010). The (un)targeted cancer kinome. *Nat Chem Biol* 6: 166–169.
- Felip E, Santarpia M, Rosell R (2007). Emerging drugs for non-small-cell lung cancer. *Expert Opin Emerg Drugs* 12: 449–460.
- Filippakopoulos P, Kofler M, Hantschel O, Gish GD, Grebien F, Salah E *et al.* (2008). Structural coupling of SH2-kinase domains links Fes and Abl substrate recognition and kinase activation. *Cell* 134: 793–803.
- Flaherty KT, Puzanov I, Kim KB, Ribas A, McArthur GA, Sosman JA *et al.* (2010). Inhibition of mutated, activated BRAF in metastatic melanoma. *N Engl J Med* 363: 809–819.
- Fleishman SJ, Schlessinger J, Ben-Tal N (2002). A putative molecular-activation switch in the transmembrane domain of erbB2. *Proc Natl Acad Sci U S A* 99: 15937–15940.
- Fletcher JA, Rubin BP (2007). KIT mutations in GIST. *Curr Opin Genet Dev* 17: 3–7.
- Force T, Krause DS, Van Etten RA (2007). Molecular mechanisms of cardiotoxicity of tyrosine kinase inhibition. *Nat Rev Cancer* 7: 332–344.
- Friboulet L, Li N, Katayama R, Lee CC, Gainor JF, Crystal AS *et al.* (2014). The ALK inhibitor ceritinib overcomes crizotinib resistance in non-small cell lung cancer. *Cancer Discov* 4: 662–673.

- Fruman DA, Rommel C (2014). PI3K and cancer: lessons, challenges and opportunities. *Nat Rev Drug Discov* 13: 140–156.
- Furman RR, Cheng S, Lu P, Setty M, Perez AR, Guo A *et al.* (2014). Ibrutinib resistance in chronic lymphocytic leukemia. *N Engl J Med* 370: 2352–2354.
- Galanis E, Buckner JC, Maurer MJ, Kreisberg JI, Ballman K, Boni J *et al.* (2005). Phase II trial of temsirolimus (CCI-779) in recurrent glioblastoma multiforme: a North Central Cancer Treatment Group Study. *J Clin Oncol* 23: 5294–5304.
- Gao Y, Davies SP, Augustin M, Woodward A, Patel UA, Kovelman R *et al.* (2013). A broad activity screen in support of a chemogenomic map for kinase signalling research and drug discovery. *Biochem J* 451: 313–328.
- Garay JP, Gray JW (2012). Omics and therapy – a basis for precision medicine. *Mol Oncol* 6: 128–139.
- Gaul MD, Guo Y, Affleck K, Cockerill GS, Gilmer TM, Griffin RJ *et al.* (2003). Discovery and biological evaluation of potent dual ErbB-2/EGFR tyrosine kinase inhibitors: 6-thiazolylquinazolines. *Bioorg Med Chem Lett* 13: 637–640.
- Glossmann H, Presek P, Eigenbrodt E (1981). Quercetin inhibits tyrosine phosphorylation by the cyclic nucleotide-independent, transforming protein kinase, pp60src. *Naunyn Schmiedebergs Arch Pharmacol* 317: 100–102.
- Goldman JM, Druker BJ (2001). Chronic myeloid leukemia: current treatment options. *Blood* 98: 2039–2042.
- Goldstein DM, Gray NS, Zarrinkar PP (2008). High-throughput kinase profiling as a platform for drug discovery. *Nat Rev Drug Discov* 7: 391–397.
- Gopal AK, Kahl BS, de Vos S, Wagner-Johnston ND, Schuster SJ, Jurczak WJ *et al.* (2014). PI3K δ inhibition by idelalisib in patients with relapsed indolent lymphoma. *N Engl J Med* 370: 1008–1018.
- Gorre ME, Mohammed M, Ellwood K, Hsu N, Paquette R, Rao PN *et al.* (2001). Clinical resistance to STI-571 cancer therapy caused by BCR-ABL gene mutation or amplification. *Science* 293: 876–880.
- Gottesman MM (2002). Mechanisms of cancer drug resistance. *Annu Rev Med* 53: 615–627.
- Graham SM, Jorgensen HG, Allan E, Pearson C, Alcorn MJ, Richmond L *et al.* (2002). Primitive, quiescent, Philadelphia-positive stem cells from patients with chronic myeloid leukemia are insensitive to STI571 *in vitro*. *Blood* 99: 319–325.
- Gray-Schopfer V, Wellbrock C, Marais R (2007). Melanoma biology and new targeted therapy. *Nature* 445: 851–857.
- Greenman C, Stephens P, Smith R, Dalgleish GL, Hunter C, Bignell G *et al.* (2007). Patterns of somatic mutation in human cancer genomes. *Nature* 446: 153–158.
- Guertin KR, Grimsby J (2006). Small molecule glucokinase activators as glucose lowering agents: a new paradigm for diabetes therapy. *Curr Med Chem* 13: 1839–1843.
- Hall MD, Handley MD, Gottesman MM (2009). Is resistance useless? Multidrug resistance and collateral sensitivity. *Trends Pharmacol Sci* 30: 546–556.
- Hall-Jackson CA, Goedert M, Hedge P, Cohen P (1999). Effect of SB 203580 on the activity of c-Raf *in vitro* and *in vivo*. *Oncogene* 18: 2047–2054.
- Hanks SK, Hunter T (1995). Protein kinases 6. The eukaryotic protein kinase superfamily: kinase (catalytic) domain structure and classification. *FASEB J* 9: 576–596.
- Hanks SK, Quinn AM, Hunter T (1988). The protein kinase family: conserved features and deduced phylogeny of the catalytic domains. *Science* 241: 42–52.
- Hardie DG (2014). AMPK: positive and negative regulation, and its role in whole-body energy homeostasis. *Curr Opin Cell Biol* 33C: 1–7.
- Harrison C, Kiladjian JJ, Al-Ali HK, Gisslinger H, Waltzman R, Stalbovskaya V *et al.* (2012). JAK inhibition with ruxotinib versus best available therapy for myelofibrosis. *N Engl J Med* 366: 787–798.
- Hatzivassiliou G, Song K, Yen I, Brandhuber BJ, Anderson DJ, Alvarado R *et al.* (2010). RAF inhibitors prime wild-type RAF to activate the MAPK pathway and enhance growth. *Nature* 464: 431–435.
- Hatzivassiliou G, Haling JR, Chen H, Song K, Price S, Heald R *et al.* (2013). Mechanism of MEK inhibition determines efficacy in mutant KRAS- versus BRAF-driven cancers. *Nature* 501: 232–236.
- Heinrich MC, Corless CL, Demetri GD, Blanke CD, von Mehren M, Joensuu H *et al.* (2003). Kinase mutations and imatinib response in patients with metastatic gastrointestinal stromal tumor. *J Clin Oncol* 21: 4342–4349.
- Heinrich T, Gradler U, Bottcher H, Blaukat A, Shutes A (2010). Allosteric IGF-1R Inhibitors. *ACS Med Chem Lett* 1: 199–203.
- Herbert C, Schieborr U, Saxena K, Juraszek J, De Smet F, Alcouffe C *et al.* (2013). Molecular mechanism of SSR128129E, an extracellularly acting, small-molecule, allosteric inhibitor of FGF receptor signaling. *Cancer Cell* 23: 489–501.
- Heymach JV, Nilsson M, Blumenschein G, Papadimitrakopoulou V, Herbst R (2006). Epidermal growth factor receptor inhibitors in development for the treatment of non-small cell lung cancer. *Clin Cancer Res* 12: 4441s–4445s.
- Hidaka H, Inagaki M, Kawamoto S, Sasaki Y (1984). Isoquinolinesulfonamides, novel and potent inhibitors of cyclic nucleotide dependent protein kinase and protein kinase C. *Biochemistry* 23: 5036–5041.
- Hill ZB, Perera BG, Andrews SS, Maly DJ (2012). Targeting diverse signaling interaction sites allows the rapid generation of bivalent kinase inhibitors. *ACS Chem Biol* 7: 487–495.
- Hindie V, Stroba A, Zhang H, Lopez-Garcia LA, Idrissova L, Zeuzem S *et al.* (2009). Structure and allosteric effects of low-molecular-weight activators on the protein kinase PDK1. *Nat Chem Biol* 5: 758–764.
- Hoesel B, Schmid JA (2013). The complexity of NF- κ B signaling in inflammation and cancer. *Mol Cancer* 12: 86.
- Holderfield M, Merritt H, Chan J, Wallroth M, Tandeske L, Zhai H *et al.* (2013). RAF inhibitors activate the MAPK pathway by relieving inhibitory autophosphorylation. *Cancer Cell* 23: 594–602.
- Hoy SM (2014). Ponatinib: a review of its use in adults with chronic myeloid leukaemia or Philadelphia chromosome-positive acute lymphoblastic leukaemia. *Drugs* 74: 793–806.
- Hsieh AC, Moasser MM (2007). Targeting HER proteins in cancer therapy and the role of the non-target HER3. *Br J Cancer* 97: 453–457.
- Hunter T (2000). Signaling – 2000 and beyond. *Cell* 100: 113–127.
- Hynes NE, Lane HA (2005). ERBB receptors and cancer: the complexity of targeted inhibitors. *Nat Rev Cancer* 5: 341–354.
- Inagaki M, Kawamoto S, Itoh H, Saitoh M, Hagiwara M, Takahashi J *et al.* (1986). Naphthalenesulfonamides as calmodulin antagonists and protein kinase inhibitors. *Mol Pharmacol* 29: 577–581.

- Jahnke W, Grotzfeld RM, Pelle X, Strauss A, Fendrich G, Cowan-Jacob SW *et al.* (2010). Binding or bending: distinction of allosteric Abl kinase agonists from antagonists by an NMR-based conformational assay. *J Am Chem Soc* 132: 7043–7048.
- Janne PA, Gray N, Settleman J (2009). Factors underlying sensitivity of cancers to small-molecule kinase inhibitors. *Nat Rev Drug Discov* 8: 709–723.
- Jia Y, Gu XJ, Brinker A, Warmuth M (2008). Measuring the tyrosine kinase activity: a review of biochemical and cellular assay technologies. *Expert Opin Drug Discov* 3: 959–978.
- Jin J, Pawson T (2012). Modular evolution of phosphorylation-based signalling systems. *Philos Trans R Soc Lond B Biol Sci* 367: 2540–2555.
- Johannessen CM, Boehm JS, Kim SY, Thomas SR, Wardwell L, Johnson LA *et al.* (2010). COT drives resistance to RAF inhibition through MAP kinase pathway reactivation. *Nature* 468: 968–972.
- Jura N, Zhang X, Endres NF, Seeliger MA, Schindler T, Kuriyan J (2011). Catalytic control in the EGF receptor and its connection to general kinase regulatory mechanisms. *Mol Cell* 42: 9–22.
- Kannan N, Taylor SS (2008). Rethinking pseudokinases. *Cell* 133: 204–205.
- Kannan N, Taylor SS, Zhai Y, Venter JC, Manning G (2007). Structural and functional diversity of the microbial kinome. *PLoS Biol* 5: e17.
- Kantarjian HM, Fojo T, Mathisen M, Zwelling LA (2013). Cancer drugs in the United States: Justum Pretium – the just price. *J Clin Oncol* 31: 3600–3604.
- Karaman MW, Herrgard S, Treiber DK, Gallant P, Atteridge CE, Campbell BT *et al.* (2008). A quantitative analysis of kinase inhibitor selectivity. *Nat Biotechnol* 26: 127–132.
- Katayama R, Shaw AT, Khan TM, Mino-Kenudson M, Solomon BJ, Halmos B *et al.* (2012). Mechanisms of acquired crizotinib resistance in ALK-rearranged lung cancers. *Sci Transl Med* 4: 120ra117.
- Kawai T, Akira S (2010). The role of pattern-recognition receptors in innate immunity: update on Toll-like receptors. *Nat Immunol* 11: 373–384.
- Kelly PA, Gruber SA, Behbod F, Kahan BD (1997). Sirolimus, a new, potent immunosuppressive agent. *Pharmacotherapy* 17: 1148–1156.
- Kennelly PJ (2002). Protein kinases and protein phosphatases in prokaryotes: a genomic perspective. *FEMS Microbiol Lett* 206: 1–8.
- Kennelly PJ (2003). Archaeal protein kinases and protein phosphatases: insights from genomics and biochemistry. *Biochem J* 370: 373–389.
- King AJ, Arnone MR, Bleam MR, Moss KG, Yang J, Fedorowicz KE *et al.* (2013). Dabrafenib; preclinical characterization, increased efficacy when combined with trametinib, while BRAF/MEK tool combination reduced skin lesions. *PLoS ONE* 8: e67583.
- Knapp S, Arruda P, Blagg J, Burley S, Drewry DH, Edwards A *et al.* (2013). A public-private partnership to unlock the untargeted kinome. *Nat Chem Biol* 9: 3–6.
- Knight JD, Pawson T, Gingras AC (2013). Profiling the kinome: current capabilities and future challenges. *J Proteomics* 81: 43–55.
- Knight ZA, Shokat KM (2005). Features of selective kinase inhibitors. *Chem Biol* 12: 621–637.
- Kobayashi S, Boggon TJ, Dayaram T, Janne PA, Kocher O, Meyerson M *et al.* (2005). EGFR mutation and resistance of non-small-cell lung cancer to gefitinib. *N Engl J Med* 352: 786–792.
- Kornev AP, Haste NM, Taylor SS, Eyck LF (2006). Surface comparison of active and inactive protein kinases identifies a conserved activation mechanism. *Proc Natl Acad Sci U S A* 103: 17783–17788.
- Kralovics R, Passamonti F, Buser AS, Teo SS, Tiedt R, Passweg JR *et al.* (2005). A gain-of-function mutation of JAK2 in myeloproliferative disorders. *N Engl J Med* 352: 1779–1790.
- Krueger DA, Care MM, Holland K, Agricola K, Tudor C, Mangeshkar P *et al.* (2010). Everolimus for subependymal giant-cell astrocytomas in tuberous sclerosis. *N Engl J Med* 363: 1801–1811.
- Kunkel GT, Maceyka M, Milstien S, Spiegel S (2013). Targeting the sphingosine-1-phosphate axis in cancer, inflammation and beyond. *Nat Rev Drug Discov* 12: 688–702.
- Kunz J, Henriquez R, Schneider U, Deuter-Reinhard M, Movva NR, Hall MN (1993). Target of rapamycin in yeast, TOR2, is an essential phosphatidylinositol kinase homolog required for G1 progression. *Cell* 73: 585–596.
- Kwak EL, Sordella R, Bell DW, Godin-Heymann N, Okimoto RA, Brannigan BW *et al.* (2005). Irreversible inhibitors of the EGF receptor may circumvent acquired resistance to gefitinib. *Proc Natl Acad Sci U S A* 102: 7665–7670.
- Lahiry P, Torkamani A, Schork NJ, Hegele RA (2010). Kinase mutations in human disease: interpreting genotype-phenotype relationships. *Nat Rev Genet* 11: 60–74.
- Lee KH, Hsu EC, Guh JH, Yang HC, Wang D, Kulp SK *et al.* (2011). Targeting energy metabolic and oncogenic signaling pathways in triple-negative breast cancer by a novel adenosine monophosphate-activated protein kinase (AMPK) activator. *J Biol Chem* 286: 39247–39258.
- Lemmon MA, Schlessinger J (2010). Cell signaling by receptor tyrosine kinases. *Cell* 141: 1117–1134.
- Levitzi A (1990). Tyrophostins – potential antiproliferative agents and novel molecular tools. *Biochem Pharmacol* 40: 913–918.
- Li B, Liu Y, Uno T, Gray N (2004). Creating chemical diversity to target protein kinases. *Comb Chem High Throughput Screen* 7: 453–472.
- Li M, Youngren JF, Mancham VP, Kozlowski M, Zhang BB, Maddux BA *et al.* (2001). Small molecule insulin receptor activators potentiate insulin action in insulin-resistant cells. *Diabetes* 50: 2323–2328.
- Lin K, Lin J, Wu W-I, Ballard J, Lee BB, Gloor SL *et al.* (2012). An ATP-site on-off switch that restricts phosphatase accessibility of akt. *Sci Signal* 5: ra37.
- Lindsley CW, Zhao Z, Leister WH, Robinson RG, Barnett SF, Defeo-Jones D *et al.* (2005). Allosteric Akt (PKB) inhibitors: discovery and SAR of isozyme selective inhibitors. *Bioorg Med Chem Lett* 15: 761–764.
- Liu Q, Sabnis Y, Zhao Z, Zhang T, Buhrlage SJ, Jones LH *et al.* (2013). Developing irreversible inhibitors of the protein kinase cysteinome. *Chem Biol* 20: 146–159.
- Liu Y, Gray NS (2006). Rational design of inhibitors that bind to inactive kinase conformations. *Nat Chem Biol* 2: 358–364.
- Logue JS, Morrison DK (2012). Complexity in the signaling network: insights from the use of targeted inhibitors in cancer therapy. *Genes Dev* 26: 641–650.
- Lombardo LJ, Lee FY, Chen P, Norris D, Barrish JC, Behnia K *et al.* (2004). Discovery of N-(2-chloro-6-methyl-phenyl)-2-(6-(4-(2-hydroxyethyl)-piperazin-1-yl)-2-methylpyrimidin-4-

- ylamino)thiazole-5-carboxamide (BMS-354825), a dual Src/Abl kinase inhibitor with potent antitumor activity in preclinical assays. *J Med Chem* 47: 6658–6661.
- London CA (2013). Kinase dysfunction and kinase inhibitors. *Vet Dermatol* 24: 181–187, e139–140.
- Lopez-Garcia LA, Schulze JO, Frohner W, Zhang H, Suss E, Weber N *et al.* (2011). Allosteric regulation of protein kinase PKC ζ by the N-terminal C1 domain and small compounds to the PIF-pocket. *Chem Biol* 18: 1463–1473.
- Lowinger TB, Riedl B, Dumas J, Smith RA (2002). Design and discovery of small molecules targeting raf-1 kinase. *Curr Pharm Des* 8: 2269–2278.
- Luo J, Solimini NL, Elledge SJ (2009). Principles of cancer therapy: oncogene and non-oncogene addiction. *Cell* 136: 823–837.
- Ma H, Deacon S, Horiuchi K (2008). The challenge of selecting protein kinase assays for lead discovery optimization. *Expert Opin Drug Discov* 3: 607–621.
- Mahon FX, Belloc F, Lagarde V, Chollet C, Moreau-Gaudry F, Reiffers J *et al.* (2003). MDR1 gene overexpression confers resistance to imatinib mesylate in leukemia cell line models. *Blood* 101: 2368–2373.
- Malumbres M, Barbacid M (2009). Cell cycle, CDKs and cancer: a changing paradigm. *Nat Rev Cancer* 9: 153–166.
- Manning G, Whyte DB, Martinez R, Hunter T, Sudarsanam S (2002). The protein kinase complement of the human genome. *Science* 298: 1912–1934.
- Martiny-Baron G, Fabbro D (2007). Classical PKC isoforms in cancer. *Pharmacol Res* 55: 477–486.
- Massa SM, Yang T, Xie Y, Shi J, Bilgen M, Joyce JN *et al.* (2010). Small molecule BDNF mimetics activate TrkB signaling and prevent neuronal degeneration in rodents. *J Clin Invest* 120: 1774–1785.
- McDermott J, Jimeno A (2014). Ibrutinib for the treatment of chronic lymphocytic leukemia and mantle cell lymphoma. *Drugs Today (Barc)* 50: 291–300.
- McIntyre KW, Shuster DJ, Gillooly KM, Dambach DM, Pattoli MA, Lu P *et al.* (2003). A highly selective inhibitor of I kappa B kinase, BMS-345541, blocks both joint inflammation and destruction in collagen-induced arthritis in mice. *Arthritis Rheum* 48: 2652–2659.
- Medina T, Amaria MN, Jimeno A (2013). Dabrafenib in the treatment of advanced melanoma. *Drugs Today (Barc)* 49: 377–385.
- Melnick JS, Janes J, Kim S, Chang JY, Sipes DG, Gunderson D *et al.* (2006). An efficient rapid system for profiling the cellular activities of molecular libraries. *Proc Natl Acad Sci U S A* 103: 3153–3158.
- Mendoza MC, Er EE, Blenis J (2011). The Ras-ERK and PI3K-mTOR pathways: cross-talk and compensation. *Trends Biochem Sci* 36: 320–328.
- Minkovsky N, Berezov A (2008). BIBW-2992, a dual receptor tyrosine kinase inhibitor for the treatment of solid tumors. *Curr Opin Investig Drugs* 9: 1336–1346.
- Moebitz H, Fabbro D (2012). Conformational bias: a key concept for protein kinase inhibition. *Eur Pharm Rev* 17: 41–51.
- Morphy R, Rankovic Z (2007). Fragments, network biology and designing multiple ligands. *Drug Discov Today* 12: 156–160.
- Motzer RJ, Hoosen S, Bello CL, Christensen JG (2006). Sunitinib malate for the treatment of solid tumours: a review of current clinical data. *Expert Opin Investig Drugs* 15: 553–561.
- Muller S, Knapp S (2010). Targeting kinases for the treatment of inflammatory diseases. *Expert Opin Drug Discov* 5: 867–881.
- Murray PJ (2007). The JAK-STAT signaling pathway: input and output integration. *J Immunol* 178: 2623–2629.
- Nelson V, Ziehr J, Agulnik M, Johnson M (2013). Afatinib: emerging next-generation tyrosine kinase inhibitor for NSCLC. *Onco Targets Ther* 6: 135–143.
- Nicolini FE, Ibrahim AR, Soverini S, Martinelli G, Muller MC, Hochhaus A *et al.* (2013). The BCR-ABL T315I mutation compromises survival in chronic phase chronic myelogenous leukemia patients resistant to tyrosine kinase inhibitors, in a matched pair analysis. *Haematologica* 98: 1510–1516.
- Ninomiya T, Takigawa N, Ichihara E, Ochi N, Murakami T, Honda Y *et al.* (2013). Afatinib prolongs survival compared with gefitinib in an epidermal growth factor receptor-driven lung cancer model. *Mol Cancer Ther* 12: 589–597.
- Nolen B, Taylor S, Ghosh G (2004). Regulation of protein kinases; controlling activity through activation segment conformation. *Mol Cell* 15: 661–675.
- Ohren JF, Chen H, Pavlovsky A, Whitehead C, Zhang E, Kuffa P *et al.* (2004). Structures of human MAP kinase kinase 1 (MEK1) and MEK2 describe novel noncompetitive kinase inhibition. *Nat Struct Mol Biol* 11: 1192–1197.
- O'Hare T, Shakespeare WC, Zhu X, Eide CA, Rivera VM, Wang F *et al.* (2009). AP24534, a pan-BCR-ABL inhibitor for chronic myeloid leukemia, potently inhibits the T315I mutant and overcomes mutation-based resistance. *Cancer Cell* 16: 401–412.
- Okuzumi T, Fiedler D, Zhang C, Gray DC, Aizenstein B, Hoffman R *et al.* (2009). Inhibitor hijacking of Akt activation. *Nat Chem Biol* 5: 484–493.
- O'Reilly T, McSheehy PM (2010). Biomarker development for the clinical activity of the mTOR inhibitor everolimus (RAD001): processes, limitations, and further proposals. *Transl Oncol* 3: 65–79.
- Over B, Wetzel S, Grutter C, Nakai Y, Renner S, Rauh D *et al.* (2013). Natural-product-derived fragments for fragment-based ligand discovery. *Nat Chem* 5: 21–28.
- Pan Z, Scheerens H, Li SJ, Schultz BE, Sprengeler PA, Burrill LC *et al.* (2007). Discovery of selective irreversible inhibitors for Bruton's tyrosine kinase. *ChemMedChem* 2: 58–61.
- Pao W, Hutchinson KE (2012). Chipping away at the lung cancer genome. *Nat Med* 18: 349–351.
- Pao W, Miller VA, Politi KA, Riely GJ, Somwar R, Zakowski MF *et al.* (2005). Acquired resistance of lung adenocarcinomas to gefitinib or erlotinib is associated with a second mutation in the EGFR kinase domain. *PLoS Med* 2: e73.
- Pargellis C, Tong L, Churchill L, Cirillo PF, Gilmore T, Graham AG *et al.* (2002). Inhibition of p38 MAP kinase by utilizing a novel allosteric binding site. *Nat Struct Biol* 9: 268–272.
- Patricelli MP, Szardenings AK, Liyanage M, Nomanbhoy TK, Wu M, Weissig H *et al.* (2007). Functional interrogation of the kinome using nucleotide acyl phosphates. *Biochemistry* 46: 350–358.
- Patricelli MP, Nomanbhoy TK, Wu J, Brown H, Zhou D, Zhang J *et al.* (2011). *In situ* kinase profiling reveals functionally relevant properties of native kinases. *Chem Biol* 18: 699–710.
- Pawson AJ, Sharman JL, Benson HE, Faccenda E, Alexander SP, Buneman OP *et al.*; NC-IUPHAR (2014). The IUPHAR/BPS Guide to PHARMACOLOGY: an expert-driven knowledgebase of drug targets and their ligands. *Nucl. Acids Res* 42 (Database Issue): D1098–D1106.

- Pender C, Goldfine ID, Mancham VP, Evans JL, Spevak WR, Shi S *et al.* (2002). Regulation of insulin receptor function by a small molecule insulin receptor activator. *J Biol Chem* 277: 43565–43571.
- Perez-Soler R (2004). The role of erlotinib (Tarceva, OSI 774) in the treatment of non-small cell lung cancer. *Clin Cancer Res* 10: 4238s–4240s.
- Plenge RM, Scolnick EM, Altshuler D (2013). Validating therapeutic targets through human genetics. *Nat Rev Drug Discov* 12: 581–594.
- Polier S, Samant RS, Clarke PA, Workman P, Prodromou C, Pearl LH (2013). ATP-competitive inhibitors block protein kinase recruitment to the Hsp90-Cdc37 system. *Nat Chem Biol* 9: 307–312.
- Poulikakos PI, Zhang C, Bollag G, Shokat KM, Rosen N (2010). RAF inhibitors transactivate RAF dimers and ERK signalling in cells with wild-type BRAF. *Nature* 464: 427–430.
- Poulikakos PI, Persaud Y, Janakiraman M, Kong X, Ng C, Moriceau G *et al.* (2011). RAF inhibitor resistance is mediated by dimerization of aberrantly spliced BRAF(V600E). *Nature* 480: 387–390.
- Prahallad A, Sun C, Huang S, Di Nicolantonio F, Salazar R, Zecchin D *et al.* (2012). Unresponsiveness of colon cancer to BRAF(V600E) inhibition through feedback activation of EGFR. *Nature* 483: 100–103.
- Quintas-Cardama A, Kantarjian H, Cortes J (2007). Flying under the radar: the new wave of BCR-ABL inhibitors. *Nat Rev Drug Discov* 6: 834–848.
- Rabindran SK, Discafani CM, Rosfjord EC, Baxter M, Floyd MB, Golas J *et al.* (2004). Antitumor activity of HKI-272, an orally active, irreversible inhibitor of the HER-2 tyrosine kinase. *Cancer Res* 64: 3958–3965.
- Rajakulendran T, Sicheri F (2010). Allosteric protein kinase regulation by pseudokinases: insights from STRAD. *Sci Signal* 3: pe8.
- Rauch J, Volinsky N, Romano D, Kolch W (2011). The secret life of kinases: functions beyond catalysis. *Cell Commun Signal* 9: 23.
- Reck M, Kaiser R, Mellemegaard A, Douillard JY, Orlov S, Krzakowski M *et al.* (2014). Docetaxel plus nintedanib versus docetaxel plus placebo in patients with previously treated non-small-cell lung cancer (LUME-Lung 1): a phase 3, double-blind, randomised controlled trial. *Lancet Oncol* 15: 143–155.
- Richeldi L, du Bois RM, Raghu G, Azuma A, Brown KK, Costabel U *et al.* (2014). Efficacy and safety of nintedanib in idiopathic pulmonary fibrosis. *N Engl J Med* 370: 2071–2082.
- Rini BI, Garrett M, Poland B, Dutcher JP, Rixe O, Wilding G *et al.* (2013). Axitinib in metastatic renal cell carcinoma: results of a pharmacokinetic and pharmacodynamic analysis. *J Clin Pharmacol* 53: 491–504.
- Robak T, Robak E (2012). Tyrosine kinase inhibitors as potential drugs for B-cell lymphoid malignancies and autoimmune disorders. *Expert Opin Investig Drugs* 21: 921–947.
- Robert C, Soria JC, Spatz A, Le Cesne A, Malka D, Pautier P *et al.* (2005). Cutaneous side-effects of kinase inhibitors and blocking antibodies. *Lancet Oncol* 6: 491–500.
- Rodon J, Dienstmann R, Serra V, Tabernero J (2013). Development of PI3K inhibitors: lessons learned from early clinical trials. *Nat Rev Clin Oncol* 10: 143–153.
- Rodrik-Outmezguine VS, Chandarlapaty S, Pagano NC, Poulikakos PI, Scaltriti M, Moskatel E *et al.* (2011). mTOR kinase inhibition causes feedback-dependent biphasic regulation of AKT signaling. *Cancer Discov* 1: 248–259.
- Rommel C (2010). Taking PI3K δ and PI3K γ one step ahead: dual active PI3K δ/γ inhibitors for the treatment of immune-mediated inflammatory diseases. *Curr Top Microbiol Immunol* 346: 279–299.
- Rommel C, Camps M, Ji H (2007). PI3K delta and PI3K gamma: partners in crime in inflammation in rheumatoid arthritis and beyond? *Nat Rev Immunol* 7: 191–201.
- Rubin BP, Duensing A (2006). Mechanisms of resistance to small molecule kinase inhibition in the treatment of solid tumors. *Lab Invest* 86: 981–986.
- Sadowsky JD, Burlingame MA, Wolan DW, McClendon CL, Jacobson MP, Wells JA (2011). Turning a protein kinase on or off from a single allosteric site via disulfide trapping. *Proc Natl Acad Sci U S A* 108: 6056–6061.
- Salama AK, Kim KB (2013). Trametinib (GSK1120212) in the treatment of melanoma. *Expert Opin Pharmacother* 14: 619–627.
- Salt IP, Palmer TM (2012). Exploiting the anti-inflammatory effects of AMP-activated protein kinase activation. *Expert Opin Investig Drugs* 21: 1155–1167.
- Sanchez-Martin M, Pandiella A (2012). Differential action of small molecule HER kinase inhibitors on receptor heterodimerization: therapeutic implications. *Int J Cancer* 131: 244–252.
- Sanders MJ, Ali ZS, Hegarty BD, Heath R, Snowden MA, Carling D (2007). Defining the mechanism of activation of AMP-activated protein kinase by the small molecule A-769662, a member of the thienopyridone family. *J Biol Chem* 282: 32539–32548.
- Sawyers C (2004). Targeted cancer therapy. *Nature* 432: 294–297.
- Scaltriti M, Verma C, Guzman M, Jimenez J, Parra JL, Pedersen K *et al.* (2009). Lapatinib, a HER2 tyrosine kinase inhibitor, induces stabilization and accumulation of HER2 and potentiates trastuzumab-dependent cell cytotoxicity. *Oncogene* 28: 803–814.
- Schmierer B, Hill CS (2007). TGF β 2-SMAD signal transduction: molecular specificity and functional flexibility. *Nat Rev Mol Cell Biol* 8: 970–982.
- Scott JD, Pawson T (2009). Cell signaling in space and time: where proteins come together and when they're apart. *Science* 326: 1220–1224.
- Sellers WR (2011). A blueprint for advancing genetics-based cancer therapy. *Cell* 147: 26–31.
- Serra V, Scaltriti M, Prudkin L, Eichhorn PJA, Ibrahim YH, Chandarlapaty S *et al.* (2011). PI3K inhibition results in enhanced HER signaling and acquired ERK dependency in HER2-overexpressing breast cancer. *Oncogene* 30: 2547–2557.
- Shahda S, Saif MW (2013). Regorafenib: from bench to bedside in colorectal cancer. *Expert Rev Clin Pharmacol* 6: 243–248.
- Shaw AT, Yasothan U, Kirkpatrick P (2011). Crizotinib. *Nat Rev Drug Discov* 10: 897–898.
- She QB, Solit D, Basso A, Moasser MM (2003). Resistance to gefitinib in PTEN-null HER-overexpressing tumor cells can be overcome through restoration of PTEN function or pharmacologic modulation of constitutive phosphatidylinositol 3'-kinase/Akt pathway signaling. *Clin Cancer Res* 9: 4340–4346.
- Shibuya M, Suzuki Y (1993). Treatment of cerebral vasospasm by a protein kinase inhibitor AT 877. *No to Shinkei* 45: 819–824.
- Shibuya M, Asano T, Sasaki Y (2001). Effect of Fasudil HCl, a protein kinase inhibitor, on cerebral vasospasm. *Acta Neurochir Suppl* 77: 201–204.
- Simmons DL (2013). Targeting kinases: a new approach to treating inflammatory rheumatic diseases. *Curr Opin Pharmacol* 13: 426–434.

- Singh J, Petter RC, Baillie TA, Whitty A (2011). The resurgence of covalent drugs. *Nat Rev Drug Discov* 10: 307–317.
- Skora L, Mestan J, Fabbro D, Jahnke W, Grzesiek S (2013). NMR reveals the allosteric opening and closing of Abelson tyrosine kinase by ATP-site and myristoyl pocket inhibitors. *Proc Natl Acad Sci U S A* 110: E4437–E4445.
- Sternberg C (2009). A randomized, double-blind phase III study of pazopanib in treatment-naïve and cytokine-pretreated patients with advanced renal cell carcinoma (RCC). ASCO, Abstract No. 5021.
- Stransky N, Cerami E, Schalm S, Kim JL, Lengauer C (2014). The landscape of kinase fusions in cancer. *Nat Commun* 5: 4846.
- Sun L, Liang C, Shirazian S, Zhou Y, Miller T, Cui J *et al.* (2003). Discovery of 5-[5-fluoro-2-oxo-1,2-dihydroindol-(3Z)-ylidenemethyl]-2,4-dimethyl-1H-pyrrole-3-carboxylic acid (2-diethylaminoethyl)amide, a novel tyrosine kinase inhibitor targeting vascular endothelial and platelet-derived growth factor receptor tyrosine kinase. *J Med Chem* 46: 1116–1119.
- Szakacs G, Paterson JK, Ludwig JA, Booth-Genthe C, Gottesman MM (2006). Targeting multidrug resistance in cancer. *Nat Rev Drug Discov* 5: 219–234.
- Takano T, Ohe Y, Sakamoto H, Tsuta K, Matsuno Y, Tateishi U *et al.* (2005). Epidermal growth factor receptor gene mutations and increased copy numbers predict gefitinib sensitivity in patients with recurrent non-small-cell lung cancer. *J Clin Oncol* 23: 6829–6837.
- Tamaoki T, Nomoto H, Takahashi I, Kato Y, Morimoto M, Tomita F (1986). Staurosporine, a potent inhibitor of phospholipid/Ca⁺⁺ dependent protein kinase. *Biochem Biophys Res Commun* 135: 397–402.
- Tao ZF, Wang L, Stewart KD, Chen Z, Gu W, Bui MH *et al.* (2007). Structure-based design, synthesis, and biological evaluation of potent and selective macrocyclic checkpoint kinase 1 inhibitors. *J Med Chem* 50: 1514–1527.
- Taylor SS, Kornev AP (2011). Protein kinases: evolution of dynamic regulatory proteins. *Trends Biochem Sci* 36: 65–77.
- Taylor SS, Kim C, Vigil D, Haste NM, Yang J, Wu J *et al.* (2005). Dynamics of signaling by PKA. *Biochim Biophys Acta* 1754: 25–37.
- Tecle H, Shao J, Li Y, Kothe M, Kazmirski S, Penzotti J *et al.* (2009). Beyond the MEK-pocket: can current MEK kinase inhibitors be utilized to synthesize novel type III NCKIs? Does the MEK-pocket exist in kinases other than MEK? *Bioorg Med Chem Lett* 19: 226–229.
- Thomas RK, Baker AC, Debiassi RM, Winckler W, Laframboise T, Lin WM *et al.* (2007). High-throughput oncogene mutation profiling in human cancer. *Nat Genet* 39: 347–351.
- Tomita N, Hayashi Y, Suzuki S, Oomori Y, Aramaki Y, Matsushita Y *et al.* (2013). Structure-based discovery of cellular-active allosteric inhibitors of FAK. *Bioorg Med Chem Lett* 23: 1779–1785.
- Traxler P, Bold G, Buchdunger E, Caravatti G, Furet P, Manley P *et al.* (2001). Tyrosine kinase inhibitors: from rational design to clinical trials. *Med Res Rev* 21: 499–512.
- Trusolino L, Bertotti A (2012). Compensatory pathways in oncogenic kinase signaling and resistance to targeted therapies: six degrees of separation. *Cancer Discov* 2: 876–880.
- Tsai J, Lee JT, Wang W, Zhang J, Cho H, Mamo S *et al.* (2008). Discovery of a selective inhibitor of oncogenic B-Raf kinase with potent antimelanoma activity. *Proc Natl Acad Sci U S A* 105: 3041–3046.
- Tummino PJ, Copeland RA (2008). Residence time of receptor-ligand complexes and its effect on biological function. *Biochemistry* 47: 5481–5492.
- Turke AB, Zejnullahu K, Wu YL, Song Y, Dias-Santagata D, Lifshits E *et al.* (2010). Preexistence and clonal selection of MET amplification in EGFR mutant NSCLC. *Cancer Cell* 17: 77–88.
- Tzarum N, Komornik N, Ben Chetrit D, Engelberg D, Livnah O (2013). DEF pocket in p38 α facilitates substrate selectivity and mediates autophosphorylation. *J Biol Chem* 288: 19537–19547.
- Ubersax JA, Ferrell JE Jr (2007). Mechanisms of specificity in protein phosphorylation. *Nat Rev Mol Cell Biol* 8: 530–541.
- Udugamasooriya DG, Dineen SP, Brekken RA, Kodadek T (2008). A peptoid ‘antibody surrogate’ that antagonizes VEGF receptor 2 activity. *J Am Chem Soc* 130: 5744–5752.
- Van Allen EM, Wagle N, Sucker A, Treacy DJ, Johannessen CM, Goetz EM *et al.* (2014). The genetic landscape of clinical resistance to RAF inhibition in metastatic melanoma. *Cancer Discov* 4: 94–109.
- Vanderpool D, Johnson TO, Ping C, Bergqvist S, Alton G, Phonephaly S *et al.* (2009). Characterization of the CHK1 allosteric inhibitor binding site. *Biochemistry* 48: 9823–9830.
- Vasquez EM (2000). Sirolimus: a new agent for prevention of renal allograft rejection. *Am J Health Syst Pharm* 57: 437–448, quiz 449–451.
- Ventura JJ, Nebreda AR (2006). Protein kinases and phosphatases as therapeutic targets in cancer. *Clin Transl Oncol* 8: 153–160.
- Vieth M, Higgs RE, Robertson DH, Shapiro M, Gragg EA, Hemmerle H (2004). Kinomics-structural biology and chemogenomics of kinase inhibitors and targets. *Biochim Biophys Acta* 1697: 243–257.
- Vieth M, Sutherland JJ, Robertson DH, Campbell RM (2005). Kinomics: characterizing the therapeutically validated kinase space. *Drug Discov Today* 10: 839–846.
- Viola D, Cappagli V, Elisei R (2013). Cabozantinib (XL184) for the treatment of locally advanced or metastatic progressive medullary thyroid cancer. *Future Oncol* 9: 1083–1092.
- Wagle N, Emery C, Berger MF, Davis MJ, Sawyer A, Pochanard P *et al.* (2011). Dissecting therapeutic resistance to RAF inhibition in melanoma by tumor genomic profiling. *J Clin Oncol* 29: 3085–3096.
- Walsh CT, Garneau-Tsodikova S, Gatto GJ Jr (2005). Protein posttranslational modifications: the chemistry of proteome diversifications. *Angew Chem Int Ed Engl* 44: 7342–7372.
- Wang X, Sun SY (2009). Enhancing mTOR-targeted cancer therapy. *Expert Opin Ther Targets* 13: 1193–1203.
- Warmuth M, Kim S, Gu XJ, Xia G, Adrian F (2007). Ba/F3 cells and their use in kinase drug discovery. *Curr Opin Oncol* 19: 55–60.
- Weinstein IB (2002). Cancer. Addiction to oncogenes – the Achilles heel of cancer. *Science* 297: 63–64.
- Weisberg E, Manley PW, Breitenstein W, Brugger J, Cowan-Jacob SW, Ray A *et al.* (2005). Characterization of AMN107, a selective inhibitor of native and mutant Bcr-Abl. *Cancer Cell* 7: 129–141.
- West K (2009). CP-690550, a JAK3 inhibitor as an immunosuppressant for the treatment of rheumatoid arthritis, transplant rejection, psoriasis and other immune-mediated disorders. *Curr Opin Investig Drugs* 10: 491–504.
- Wiestner A (2013). Targeting B-cell receptor signaling for anticancer therapy: the Bruton’s tyrosine kinase inhibitor ibrutinib induces impressive responses in B-cell malignancies. *J Clin Oncol* 31: 128–130.
- Williams NK, Bamert RS, Patel O, Wang C, Walden PM, Wilks AF *et al.* (2009). Dissecting specificity in the Janus kinases: the

structures of JAK-specific inhibitors complexed to the JAK1 and JAK2 protein tyrosine kinase domains. *J Mol Biol* 387: 219–232.

Wissner A, Fraser HL, Ingalls CL, Dushin RG, Floyd MB, Cheung K *et al.* (2007). Dual irreversible kinase inhibitors: quinazoline-based inhibitors incorporating two independent reactive centers with each targeting different cysteine residues in the kinase domains of EGFR and VEGFR-2. *Bioorg Med Chem* 15: 3635–3648.

Wolf-Yadlin A, Kumar N, Zhang Y, Hautaniemi S, Zaman M, Kim HD *et al.* (2006). Effects of HER2 overexpression on cell signaling networks governing proliferation and migration. *Mol Syst Biol* 2: 54.

Wood ER, Truesdale AT, McDonald OB, Yuan D, Hassell A, Dickerson SH *et al.* (2004). A unique structure for epidermal growth factor receptor bound to GW572016 (Lapatinib): relationships among protein conformation, inhibitor off-rate, and receptor activity in tumor cells. *Cancer Res* 64: 6652–6659.

Workman P, Al-Lazikani B (2013a). Drugging cancer genomes. *Nat Rev Drug Discov* 12: 889–890.

Workman P, Al-Lazikani B, Clarke PA (2013b). Genome-based cancer therapeutics: targets, kinase drug resistance and future strategies for precision oncology. *Curr Opin Pharmacol* 13: 486–496.

Wright CJ, McCormack PL (2013). Trametinib: first global approval. *Drugs* 73: 1245–1254.

Wymann MP, Bulgarelli-Leva G, Zvelebil MJ, Pirola L, Vanhaesebroeck B, Waterfield MD *et al.* (1996). Wortmannin inactivates phosphoinositide 3-kinase by covalent modification of Lys-802, a residue involved in the phosphate transfer reaction. *Mol Cell Biol* 16: 1722–1733.

Yang H, Rudge DG, Koos JD, Vaidialingam B, Yang HJ, Pavletich NP (2013). mTOR kinase structure, mechanism and regulation. *Nature* 497: 217–223.

Yang JC (2013). A selective ALK inhibitor in ALK-rearranged patients. *Lancet Oncol* 14: 564–565.

Yang J, Campobasso N, Biju MP, Fisher K, Pan XQ, Cottom J *et al.* (2011). Discovery and characterization of a cell-permeable, small-molecule c-Abl kinase activator that binds to the myristoyl binding site. *Chem Biol* 18: 177–186.

Yang X, Huang Y, Crowson M, Li J, Maitland ML, Lussier YA (2010). Kinase inhibition-related adverse events predicted from in vitro kinome and clinical trial data. *J Biomed Inform* 43: 376–384.

Yuan TL, Cantley LC (2008). PI3K pathway alterations in cancer: variations on a theme. *Oncogene* 27: 5497–5510.

Zeqiraj E, Filippi BM, Deak M, Alessi DR, van Aalten DM (2009). Structure of the LKB1-STRAD-MO25 complex reveals an allosteric mechanism of kinase activation. *Science* 326: 1707–1711.

Zhang J, Yang PL, Gray NS (2009). Targeting cancer with small molecule kinase inhibitors. *Nat Rev Cancer* 9: 28–39.

Zhang J, Adrian FJ, Jahnke W, Cowan-Jacob SW, Li AG, Iacob RE *et al.* (2010). Targeting Bcr-Abl by combining allosteric with ATP-binding-site inhibitors. *Nature* 463: 501–506.

Zhou W, Ercan D, Chen L, Yun CH, Li D, Capelletti M *et al.* (2009). Novel mutant-selective EGFR kinase inhibitors against EGFR T790M. *Nature* 462: 1070–1074.

Zhou W, Ercan D, Janne PA, Gray NS (2011). Discovery of selective irreversible inhibitors for EGFR-T790M. *ACS Med Chem Lett* 21: 638–643.

Zuccotto F, Ardini E, Casale E, Angiolini M (2010). Through the 'gatekeeper door': exploiting the active kinase conformation. *J Med Chem* 53: 2681–2694.