New perspectives, opportunities and challenges in exploring the human protein kinome

Leah J. Wilson[1], Adam Linley[2], Dean E. Hammond[1], Fiona E. Hood[1], Judy M. Coulson[1], David J. MacEwan[3], Sarah J. Ross[4], Joseph R. Slupsky[2], Paul D. Smith[4], Patrick A. Eyers[5] and Ian A. Prior[1,6]

CAN-17-2291R

Running Title: Human protein kinome knowledgebase
Keywords: Protein kinase, pseudokinase, phosphorylation, signaling, disease

Word Count 6402 + Figure Legends + References

[1] Department of Cellular and Molecular Physiology, Institute of Translational Medicine, University of Liverpool, L69 3BX, UK.

[2] Department of Clinical Cancer Medicine, Institute of Translational Medicine, University of Liverpool, L69 3GE, UK.

[3] Department of Molecular and Clinical Pharmacology, Institute of Translational Medicine, University of Liverpool, L69 3GE, UK.

[4] AstraZeneca, Cambridge Biomedical Campus, Cambridge, CB2 0AA, UK.

[5] Department of Biochemistry, Institute of Integrative Biology, University of Liverpool, L69 7ZB, UK.

[6] Corresponding Author: Ian A. Prior
Email: iprior@liverpool.ac.uk
Tel: +44-151-794-5332
Fax: +44-151-794-4434

ABSTRACT

The human protein kinome comprises 535 proteins that, with the exception of ~50 pseudokinases, control intracellular signaling networks by catalyzing the phosphorylation of multiple protein substrates. Whilst a major research focus of the last 30 years has been cancer-associated Tyr and Ser/Thr kinases, over 85% of the kinome has been identified to be dysregulated in at least one disease or developmental disorder. Despite this remarkable statistic, for the majority of protein kinases and pseudokinases there are currently no inhibitors progressing towards the clinic, and in most cases, details of their physiological and pathological mechanisms remain at least partially obscure. By curating and annotating data from the literature and major public databases of phosphorylation sites, kinases and disease associations, we generate an unbiased resource that highlights areas of unmet need within the kinome. We discuss strategies and challenges associated with characterising catalytic and non-catalytic outputs in cells, and describe successes and new frontiers that will support more comprehensive cancer-targeting and therapeutic evaluation in the future.

INTRODUCTION

Protein kinases, which are nearly all members of the eukaryotic protein kinase (ePK) superfamily, represent a large and diverse family of enzymes that catalyze the context-dependent transfer of the γ–phosphate of ATP onto specific protein substrates. Modulation of protein function by kinase-mediated phosphorylation of alcoholic amino acid side chains (Ser, Thr and Tyr) underpins much of biological signaling, and kinase dysregulation is frequently associated with disease. Consequently, this protein superfamily has been the subject of increasingly intensive scrutiny ever since the first protein kinase activity (phosphorylase kinase) was characterized by Krebs and Fischer in 1955 (1).

The first comprehensive survey of the human kinase complement by Manning and colleagues identified and classified 518 protein kinases, by grouping them into evolutionary-related families based on statistical sequence analysis (2). Since publication of this groundbreaking census, further kinome-wide appraisal has been undertaken from a variety of research angles (3-6). With recent estimates suggesting that phosphorylation occurs on ~90% of proteins expressed in cultured human cells (7), the contemporary relevance of kinome-wide analysis remains extremely high. Furthermore, a recent wide-ranging protein phosphatase census confirms the presence of 189 distinct human protein phosphatase genes (8). Together, protein kinases and phosphatases constitute an important regulatory force in signaling whose unequivocal medical relevance has now led to decades of successful pharmacological intervention (9). Important recent data also reveals widespread Histidine phosphorylation in human cells, likely catalyzed by NME1 and 2 at chemically distinct 1 and 3 positions of the imidazole ring to form chemically-labile phosphoramidate bonds (10-12). This development implies the need for further technological innovation in order to more comprehensively evaluate non-classical cellular phosphorylation, whilst providing a timely reminder of the need for an unbiased analysis of poorly studied members of the human kinome to be prioritized. This will be important to evaluate whether some of the newly annotated members of the kinome eg. NME3-9 are *bona fide* protein kinases or pseudokinases.

To support kinome analysis, several databases and on-line tools have been designed to take advantage of the significant developments in mass spectrometry-based technology and technical advances in kinase-substrate identification (13,14). Together, these now permit deeper knowledge of various aspects of kinase biology to be compiled and connected. However, a key issue for both expert and non-expert users of such databases is a general lack of kinase naming conformity, which does not permit easy comparative kinome analysis.

Up-to-date information on kinome physiology, disease association and progress in therapeutic targeting can readily be obtained from public databases (Figure 1, Table 1, Supplementary Table 1). Such resources can also be mined to evaluate specialised 'niche' kinome data that might be important for rarer cancers, a recent example being the complex cellular landscape of mitosis-specific phospho-tyrosine (15). In this resource-based review we have curated major insights from these sources to provide a current, readily accessible, overview of important aspects of human kinome biology.

KINOME BIOLOGY

The human kinome consists of 535 distinct protein kinases (Supplementary Table 1; KinBase: www.kinase.com). 479 kinases contain a recognized ePK catalytic domain, which can be further sub-classified based on primary sequence into seven major ePK families: TK, TKL, STE, CK1, AGC, CAMK and CMGC (2). 81 of the ePK superfamily represent sub-branches of the kinome dendrogram that do not fit within the seven major groups and are classified as 'Other' (2). The RGC kinase family, included in Figure 1 and our datasets, has recently been re-classified as a sub-group within the 'Other' family (KinBase: www.kinase.com). The remaining 56 non-ePK kinases within the kinome possess an atypical protein kinase domain that has little sequence similarity to the main kinase superfamily, and their classification into distinct kinase sub-families is probably more appropriate (16). However, proteins within this Atypical group have verified, or are predicted to maintain, kinase activity based on biochemical experiments and/or structural analysis (2). Intriguingly, both the Atypical and Other kinase families have an over-representation of kinases shown to be essential in at least 6 of the 11 cell lines used across three genome-wide studies of essential genes (Figure 1) (17-19), in broad agreement with earlier unbiased pioneering studies comparing siRNA-based near kinome-wide knockdown across human cell lines (20). Finally,

some 52 kinase family members are believed to lack the appropriate catalytic machinery to efficiently phosphorylate standard substrates. These pseudokinases are distributed across all of the families of the kinome (Figure 1), suggesting that an absence of catalysis is not a formal barrier to the evolution of unique or irreplaceable biological roles, nor the acquisition of cancer-associated signaling functions. Moreover, the existence of pseudokinases within the kinomes of all eukaryotic organisms analyzed argues for increasingly nuanced evaluation procedures when the biological roles of kinase-dependent and independent functions of all kinome members are assessed experimentally.

Deep proteomic analysis of 23 different mammalian cell lines has revealed that cells often contain at least 300 different kinases (21-25). However, the overview of published data (Figure 1) illustrates that the major research focus has been on tyrosine kinases (TK) and a select few other kinases that are critical for promoting cell proliferation and survival. For example, the 12 principle kinase nodes within the EGFR/ERBB2-MAP kinase signaling network together with AKT family members account for almost 20% of the ~120,000 kinome publications. In contrast, half of the kinome still only accounts for only ~5% of research publications (Supplementary Table 1). The kinases that have been most studied nearly all have conserved, rate-limiting, roles in normal vertebrate cell biology and exhibit significant associations with diseases and/or developmental disorders, which has helped prioritize their pharmaceutical evaluation. Consequently, most have now been successfully targeted by chemical inhibitors that have secured FDA approval or reached an advanced stage of clinical trial.

The 90 tyrosine kinases are particularly well served by FDA approved inhibitors (Figure 1). However, many of these compounds exhibit very broad specificity, including frequent nM inhibitory potencies for ‘off-target’ kinases lying outside of the TK family (Figure 1; Supplementary Tables 1 & 2). Amongst the non-TK families, only BRAF, MEK1, MEK2,

CDK4/CDK6 and mTOR have (knowingly) had drugs specifically developed towards them that received FDA approval as of July 2017 (Table 1). An overt focus on the kinases known to play critical roles in cancer etiology suggests that it is likely that the development of drugs (or even specific tool compounds) for the majority of the kinome still lie at an early stage in development. Although this issue has been discussed previously (4), some 300 members of the kinome still do not have any inhibitors that have entered a clinical trial and >200 do not have any publicly available structural information available to assist in drug design (Figure 1, Supplementary Table 1). This is an important area of unmet need, because the availability of selective inhibitors has a significant impact on understanding the function of the target kinase. Integration of text mining, manually curated disease-gene association databases, cancer mutation data and genome-wide association studies reveals that >85% of the kinome is associated with at least one disease or developmental disorder which can arguably only be best addressed or validated by the use of selective inhibitors (Supplementary Table 1; http://diseases.jensenlab.org; (26,27)).

ACTIVE KINASES AND PSEUDOKINASES

Protein kinase domains consist of a small N-terminal lobe that is predominantly responsible for co-ordinating ATP binding and a large C-terminal lobe that makes a major contribution to protein substrate binding and catalysis of phosphorylation (28). The mechanistic basis for the process of phosphorylation by kinases is described in detail elsewhere (29). Regulation of protein kinase activity occurs *via* multiple post-translational modifications (PTMs; most notably phosphorylation), auto-inhibition, binding to a regulatory partner, which can include both activator and inhibitor proteins and/or changes in expression (29,30). One or more of these mechanisms are employed by most kinases to promote or stabilize an active conformation and support ATP and substrate binding capabilities of the protein kinase domain (28).

Non-enzymatic members of the human kinome, exemplified by the pseudokinases, have now emerged as important areas of fundamental research. ~50 human pseudokinases (31) have been catalogued and assigned to the pseudokinase group, consistently representing ~10% of genes found in vertebrate kinomes (Supplementary Table 1) (2,32). Despite exhibiting low or zero levels of catalytic output when assayed biochemically, pseudokinases can sometimes still bind physiological concentrations of nucleotides and so retain the ability to act as molecular signaling switches functioning in cells through druggable ligand-induced transitions that are of particular interest for pharmaceutical-design approaches (33,34). Pseudokinases can also actively control the catalytic output of enzymes by either allosteric modulation, competition for substrate binding, re-localization of active partner enzymes, or *via* scaffolding and integration of distinct signaling pathways (32). Prominent examples are HER3/ERBB3, which is a major HER2 signaling partner in tumor cells, and a central modulator of cancer cell drug-resistance that acts as a scaffold to induce and maintain PtdIns-3-kinase (PI3K) activity (35,36) and KSR1 and KSR2 in the EGFR-Ras-MAP kinase pathway which act as scaffolds to regulate the signaling activity, through allosteric interactions, of their respective catalytically-active RAF relatives (37,38). Data mining has confirmed that mutated or overexpressed pseudokinases are associated with many human diseases, including cancers (32,39). A major challenge in the future will be to harness the insights from the development of clinical kinase inhibitors to target the wide range of atypical conformations that define disease-associated variants of pseudokinases and signaling-active, but enzymatically inactive, canonical kinases.

The non-enzymatic mechanisms of pseudokinase regulation of kinase partner protein function are also exhibited by catalytically active kinases such as RAF and AURKA (40), and this should be borne in mind when seeking to understand responses to targeted therapies. For example, RAF inhibitors can in certain cellular contexts promote transactivation of RAF

dimers and explain paradoxical activation of RAF signaling in cells (41,42). It remains likely that non-enzymatic mechanisms of signaling are often unknowingly being drugged with clinical agents; a further key goal for the future will be to establish the contribution of inactive and non-enzyme mechanisms to signaling, and to target them more appropriately in patients.

PROTEIN PHOSPHORYLATION

The expansion of the 'basic' cellular proteome configuration (43) through reversible multi-site protein phosphorylation constitutes an enormous challenge for the rapidly maturing phospho-proteomics field. Almost 250,000 human Ser/Thr/Tyr phospho-sites have now been experimentally identified and curated from the available proteomic literature and in-house phospho-proteomic datasets by PhosphoSitePlus® (Figure 2; www.phosphosite.org; (44)). A typical cell might in fact contain twice this number of modified residues (7,45) and we are now in a strong position to interpret this information in terms of cell physiology. Advances in quantitative experimental strategies, sample methodologies and targeted mass spectrometric sensitivity mean that in a typical experiment >10,000 phospho-sites can routinely be identified from low milligram quantities of starting material (46). The most commonly used enrichment strategies use metal oxides such as TiO_2, which are highly specific for most phosphopeptides (47). However, such approaches can result in relatively poor sampling of the phospho-tyrosine (pTyr) pool; therefore, anti-pTyr antibody-based enrichment is typically employed to evaluate this less-abundant modification (47). Effective sampling of this subset is particularly important given the dominant role of tyrosine kinases in controlling early events in signaling that are frequently dysregulated in diseases such as cancer (48,49). An important technical challenge will be the development of advanced analytical approaches to sample the extent and positional distribution of acid-labile, rare, sub-stoichiometric and combinatorial phosphorylation in human cells. For example, site-specific His phosphorylation in human cells

has only recently been recognized experimentally (12). Analogous to the importance of pTyr antibodies in the race to decode the biological role of pTyr, the availability of high-affinity monoclonal antibodies targeting 1 and 3-phosphorylated His (11) and improved mass spectrometry workflows (50) have significant potential to simplify this challenge. Further targeted and discovery-based proteomics approaches will also be critical to understand how combinations of PTMs together make up signaling codes and can be successfully targeted for therapeutic intervention.

5-20% of phospho-sites exhibit regulated changes in large scale experiments (51-53), whilst fewer than 2% (5330 phospho-sites) have known regulatory consequences for their target proteins (44). This primitive understanding about the functional consequences, or stoichiometry, of >98% of phosphorylation extends to challenging questions about whether low level 'noise' in signaling is unimportant for systems-level analysis because it is driven by inefficient protein kinase enzymology. This illustrates the scale of the challenge for generating broad mechanistic insight from phospho-proteomic datasets. Indeed, an important regulatory target of kinase activity is other protein kinases; 993 of the curated regulatory phospho-sites are found on kinases and there is a clear enrichment for pTyr in regulating enzyme activity (Figure 2). Details of known regulatory kinase phosphorylation sites are provided in Supplementary Table 1. Other major regulatory functions of phosphorylation are in modulation of localization, interactions and protein stability to influence the dynamics and context of protein function (Figure 2) and all are suitable for therapeutic manipulation (54-56).

KINASE-SUBSTRATE RELATIONSHIPS

Figure 2 and Supplementary Table 3 curate 301 experimentally determined protein kinase consensus sequences highlighting the five broad categories of kinase recognition motifs utilizing combinations of acidic, basic, hydrophobic, Pro and pre-phosphorylated Ser or Thr

residues adjacent to the target residue (57,58). In general, many tyrosine kinases prefer adjacent acidic and hydrophobic residues whilst Ser/Thr kinases typically phosphorylate residues adjacent to basic motifs or Pro residues. However, it is important to note that not all substrates contain linear consensus sequences and instead rely on non-contiguous sequence being brought together during protein folding or after conformational changes (59). The extent to which different combinations of PTMs might change kinase or phosphatase substrate specificity is also unclear and concerted effort to understand the co-existence and combinatorial regulation of PTMs in cells remains a high priority for the kinome field (60,61).

Phospho-proteomic analysis will be a key driver of knowledge in this area. In order to support rapid 'first pass' analysis of phospho-proteomic datasets we have generated an instructive phospho-proteome profiler that provides an overview of potential kinase regulators of specific phospho-sites and highlights all known regulatory and disease associated sites within a submitted dataset (Supplementary File 1). For example, included within the phospho-proteome profiler are 20,266 experimentally verified kinase-substrate relationships curated from major studies (44,58,62-65). Interestingly, 80% of kinases within the dataset phosphorylate ≤50 substrates and 90% of phospho-sites are targets of ≤6 kinases. Whilst some kinases, such as the dual specificity kinases MAP2K1/MAP2K2, are believed to have a very restricted substrate pool, in general these numbers are certainly significant underestimates of cellular kinase activity since <5% of known phospho-sites and <80% of kinases are included within the dataset. This reflects the sampling bias due to the focus of most studies on particular members of the kinome (highlighted in Figure 1). An example of how extensive the substrate pool could be for many kinases is seen with the very well-studied MAPK1 for which over 850 substrates have already been identified (Supplementary File 1).

To circumvent the paucity of coverage of kinase-substrate relationships, researchers have focused on predictive tools based on kinase consensus motifs and other contextual

information to infer putative kinase regulators of phospho-sites (65-75). Cellular context is particularly important when predicting signaling interactions, yet this is rarely included in database annotations with the notable exception of the PHOSIDA database (76). This is now starting to change and the latest iterations of predictive tools integrate dynamic changes in phospho-proteome or interactome data with kinase consensus motif information to improve predictions of likely kinase regulators (66,77).

Characterizing dynamic changes in kinome activity is necessary to understand network contributions to normal cell activity or rewiring in response to therapeutic interventions. The occupancy of phosphorylation often changes markedly and rapidly when unstimulated and stimulated cells are compared side-by-side (7,78). Whilst *in vitro* kinome profiling is extensively used for assessing drug specificity and sensitivity (79), the capacity for cellular kinome profiling remains much more challenging at the proteomic level. Most studies utilize combinations of gene expression profiling, gene set enrichment analysis, kinome-wide chemo-genetic screens, reverse phase protein arrays or kinase antibody arrays to infer changes in kinase activity and network responses. Recently developed proteomic approaches offer some interesting complementary alternatives. Quantotypic peptides have been identified that allow accurate quantitation of the relative protein expression levels of ~20% of the kinome (80). Broad-spectrum kinase inhibitors immobilized on beads can be used to enrich kinases from cell lysates for proteomic analysis and relative profiling of protein expression levels or drug sensitivity (81-86). This approach has been claimed to be sensitive to kinase activation state across at least 75% of kinome and tyrosine kinases mediating drug resistance in cancer have been identified using this method (87-90). However, whilst it is likely able to report the protein expression levels of many kinases, the ability to differentially enrich for active versus inactive kinases is likely to be highly context dependent and has not yet been formally verified beyond a small number of well-studied tyrosine kinases (91).

Finally, the integration of proteomics and large scale kinase activity screening approaches with genomic and transcriptomic datasets is essential for systems-level understanding of kinome networks and their contributions to normal biology and diseases such as cancer (92-94). The need for improved computational methods for integrating and deciphering the multi-omic cancer datasets has been recognized by recent proteogenomics funding initiatives from the National Cancer Institute and others. Whilst the paucity of understanding of node regulation and biological consequences across kinome networks described above illustrates the scale of the challenge, multi-'omics analysis and systems levels understanding will be critical for developing personalized medicine approaches.

KINOME DISEASE ASSOCIATION

Over 450 kinases have been implicated in the development or progression of diseases (26). Notably, 448 of these have been linked to various genetic and signaling cancer hallmarks, whilst 230 potentially play a role in the development of other diseases and developmental disorders (Supplementary Table 1). Examples where gain or loss of kinase function might underlie 'non-cancer' diseases include PINK1 and LRRK2, which function in mitophagy pathways associated with Parkinson's disease (95). DYRK1A catalytic activity is required for neuronal development and overexpression is associated with Down's syndrome whilst haplo-insufficiency causes microcephaly (96). Truncating and missense mutations of TTN cause cardiomyopathy (97), whilst deletion of the FAM20C gene (the *bona-fide* 'casein kinase' responsible for generating the phosphorylated secretome) results in bone dysplasia due to loss of phosphorylation of extracellular proteins required for biomineralization (98). However, the causal role of the majority of kinases in specific diseases is unclear even when considering the roles of human kinases in cancer, where most attention has been focused. For example, whilst the Sanger Cancer Gene Census (CGC) identified the kinase domain as

the most frequently encoded domain in ‘cancer genes’ (99), the CGC database currently identifies only 58 kinases where mutations are causally associated with cancer.

The role of mutations or copy number changes as drivers or passengers in disease can be hard to discern particularly when mutated at low frequency. The advent of widespread cancer genome sequencing has provided large datasets that, together with statistical analysis that accounts for mutational heterogeneity, means cancer drivers can be more accurately identified (100,101). MutSigCV analysis measures whether the observed mutation frequency for a given gene differs from background rates for the cancer type and the local sequence context (100). These data together with measurements of copy number alterations (CNA) are collated on The Cancer Genome Atlas (TCGA; http://cancergenome.nih.gov/) and the kinome subset is curated in Supplementary Table 4.

122 kinases out of a total pool of 3341 genes exhibit significant mutation rates ($q \leq 0.1$, Benjamini-Hochberg; MutSigCV) and/or copy number changes (≥5% patients) in at least one of the 25 TCGA cancer datasets studied. Tyrosine kinases and TKL family members are over-represented amongst the significantly mutated subset comprising 67 kinases that contribute half of all of the observations across the tumor types (Figure 3; Supplementary Table 4). Importantly, due to the incomplete understanding of kinome biology, we have not been able to comprehensively discriminate between gain or loss of function mutations or silent mutations and therefore present the gross rates rather than values adjusted for known functional effects.

A selection of the most mutated or copy number altered kinases are depicted in Figure 3. The heterogeneous mutational/CNA landscape across tumor types becomes apparent with, for example, kidney cancers (KIRC and KIRP) harboring very few mutations whereas lung adenocarcinoma (LUAD) exhibits high rates of mutation and copy number variations. With the exception of a few well-known oncogenes such as BRAF, KIT, EGFR and FLT3, kinases are typically significantly mutated at low frequencies. However, tumors can contain combinations

from a selection of up to 58 different kinases exhibiting significant rates of mutation compared to background. Notably, important kinase effectors of oncogenic pathways such as AKT2, MAP2K1, MAPK1 and MAPK3 are rarely mutated above background levels despite central rate-limiting roles in proliferation and apoptosis, illustrating the focus of most perturbations on kinases initiating network responses.

The role of copy number alterations as cancer drivers or passengers is even more challenging to ascertain. Confounding factors include the focal nature of the amplicon and heterogeneity of the amplification ie not all regions of the tumour are amplified (102). This means that amplification as biomarkers for patient selection are not binary like point mutations. A *bona fide* example of cancer driving amplification is observed with ERBB2, which contributes to an aggressive phenotype in breast cancer (103). However, there are 11 other kinases that show equivalent or greater levels of amplification in the BRCA dataset that have not been evaluated for their contribution to breast cancer. Similarly, MAP3K13, PRKCI and PTK2 show high frequencies of amplification in a broad cross-section of cancers that most likely reflects their genomic positions adjacent to frequently amplified oncogenes such as MYC and PIK3CA rather than a direct cancer role. Despite these challenges, potentially intriguing patterns of copy number change are observed. For example, the specific amplifications of WNK1 in testicular cancer (TGCT) and PIM1 in uveal melanoma (UVM) are observed against backgrounds of exceptionally low rates of genetic alterations (Figure 3). These are analogous to the patterns of mutation seen with driver oncogenes such as FLT3 in acute myeloid leukemia (LAML; (104)), and may indicate a specific driver contribution in these cancer types.

To summarize, whilst 122 kinases exhibit significant rates of mutation and 78 kinases exhibit appreciable levels of copy number alterations (≥5% of patients) in at least one of the broad cross-section of cancer types in our dataset, only a subset of these are likely to reflect

a direct ‘driver’ contribution. The lack of certainty even when considering something as intensively studied as human cancer signaling, most likely reflects the relentless focus on only a subset of commonly mutated or studied kinases and the general lack of understanding of the full consequences of gain or loss of canonical functions of a kinase. The challenge here is exemplified by recent studies that showed that kinase-dead BRAF and loss of function mutations in PKC are both oncogenic (105,106). Although we may well have identified many of the major kinase players in human primary cancers, in order to fully develop personalized medicine approaches the contributory role of all kinases in patient sub-populations will have to be fully characterized. This will include improved focus on kinases that currently lack good chemical biology tools, and on validating and modulating protein kinases that drive metastatic programming. Similarly, there are many key regulators of kinase biology (eg. KRAS, PTEN, PIK3CB, GNAQ, NF1) that represent challenging but important contemporary targets or biomarkers for therapeutic development. The importance of targeting dysregulated or mutated protein kinases in diseases with the highest levels of human morbidity such as heart disease, chronic obstructive pulmonary disorder, acute infection and dementia should also not be forgotten.

KINOME THERAPEUTICS

Although kinases are targets for ~15% of the compounds collated in ChEMBL v.21, they currently represent fewer than 5% of the almost 1600 drugs that have received FDA approval to date (3,107,108). Interestingly, 235 kinases are established primary targets of inhibitors that have entered Phase 1 clinical trials. A further 127 kinases are known targets of experimental compounds that broadly satisfy Lipinski’s principles, that some, but not all kinase-directed small molecules obey ((109); Supplementary Table 2). The first protein kinase

inhibitor to be approved for treatment was the ROCK inhibitor Fasudil in 1995 in Japan and China; however, it was a further 4 years before the mechanistically unique mMTORC1 complex inhibitor sirolimus, also known as rapamycin (110), became the first kinase inhibitor to receive FDA approval. Since 1999, and spurred-on by the breakthrough efficacy of imatinib in chronic myeloid leukemia (CML) and then gastro-intestinal stromal tumor (GIST) patients, a further 35 small molecule kinase inhibitors have received FDA approval as of August 2017 (Figure 4). The majority target tyrosine kinases and are prescribed for cancer therapeutics, although oral dual JAK1/2 inhibitors bucked this trend, following approval of ruxolitinib for myelofibrosis in 2015 and tofacitinib for rheumatoid arthritis in 2016 (111). A second group of agents targeting kinases are represented by humanised monoclonal antibodies, which target the extracellular domains of receptor tyrosine kinases to prevent ligand binding and/or promote antibody-dependent immune cell-mediated toxicity (112). Antibody-mediated approaches to kinome therapeutics are likely to define the continuing marriage of technology with biologics, encompassing combination antibody therapies with small molecule kinase inhibitors (113). The final group of current kinome therapeutics comprise the ligands and ligand modulators. The only member of this group that does not target a receptor tyrosine kinase is linaclotide, a peptide ligand of the pseudokinase-containing guanylate cyclase GUCY2C, which is used to treat irritable bowel syndrome (114). Ligand modulators exclusively consist of VEGF antagonists that oppose the angiogenesis promoting activity of the VEGFR pathway (115). These RNA-aptamer and protein-based antagonists of VEGF have successfully been used since 2004 for treating cancer and ocular vascular disease.

An important feature of many chemical inhibitors of kinases is their relative lack of single kinase selectivity. 170 members of the kinome have sub-100 nM sensitivity to at least one FDA approved drug (Figures 1, 4, 5 and Supplementary Table 2), and this is particularly evident with respect to the SRC family kinase inhibitors dasatinib, bosutinib and nintedanib

that possess an inhibitory spectrum far beyond their ‘target; kinases (Figure 5). One reason for this lack of selectivity is that the ATP-binding site is highly conserved between kinases and therefore can represent a promiscuous target especially for ATP-competitive Type I inhibitors (comprising many FDA approved small molecule kinase inhibitors) that bind the active kinase. These ‘off-target’ effects are not limited to protein kinases, and include interesting targets such as bromodomain and extra-terminal domain (BET) family proteins (116), the haem biosynthetic enzyme ferrochelatase (86,117) and a variety of other ATP-binding proteins (86,118). In contrast, although Type II inhibitors (eg. imatinib, sorafenib, regorafenib) that stabilize the inactive kinase conformation are still somewhat promiscuous as a class (119), the potential to select for fewer ‘inactive’ conformations amongst their intracellular targets does exist (3). Improvements in medicinal chemistry, understanding of structure-activity relationships and selectivity screening mean that much more selective ATP-competitive kinase inhibitors are being developed. These include approved EGFR tyrosine kinase inhibitors and many more drugs in clinical development. The highest levels of cellular specificity are observed with Type III inhibitors that target signaling *via* allosteric mechanisms (120). Examples of Type III inhibitors include the MEK1/2 inhibitors trametinib, cobimetinib and selumetinib (granted FDA orphan drug designation in 2016 for treatment of advanced thyroid cancer) (121). The availability of allosteric and catalytic site kinase inhibitors presents the opportunity for dual hit inhibition where the distinct modes of drug action are thought to enhance target coverage and reduce the emergence of drug resistance. A successful application of this strategy has been seen with BCR-ABL inhibitor nilotinib in combination with the allosteric inhibitor ABL001 where stereotypical drug resistance in the BCR-ABL target failed to emerge in pre-clinical leukemia models (122). The potential benefits of allosteric inhibitors means that their discovery and development should remain a major focus for research efforts.

The extent of specificity amongst the kinase inhibitors obviously has implications for treatment and personalized medicine. Many tyrosine kinase inhibitors potentially have broad specificity at clinical doses (Figure 5), which means that multiple kinase nodes within an oncogenic pathway may be beneficially targeted through intentional 'polypharmacology' (123). However, the ability to inhibit the desired, disease driving, kinase target optimally without being limited by toxicity due to polypharmacology is very important. A case in point is that many kinase inhibitors exhibit cardiotoxicity through induction of long QT syndrome (124). The publicly available data to assess target specificity of chemical inhibitors is not comprehensive. For example, nine of the inhibitors have available test data against fewer than twenty kinases (Supplementary Table 2). Consequently, some of the drugs that appear to be selective may target a wider range of kinase conformations than indicated (Figure 5), especially given that structurally-distinct kinase and pseudokinase families are usually absent from screening platforms, despite their potentially druggable links to various cancer phenotypes.

ACQUIRED RESISTANCE AND ADAPTIVE KINOME REPROGRAMMING.

Despite the successes in small molecule kinase inhibitor development, resistance to therapy frequently occurs and most patients eventually relapse. The mechanisms by which tumors can acquire resistance to kinase inhibitors is complex. Two broad mechanisms are responsible for the development of resistance following response to therapy: an adaptive phase where signaling pathways can be remodeled to mitigate the effects of kinase inhibition and a longer-term process where mutations or gene copy number alterations may be acquired that confer a selective advantage by resisting the effects of the treatment. The relative contribution of these mechanisms to resistance varies greatly between different kinase drug targets.

Adaptive resistance makes use of existing homeostatic feed-forward and feedback loops to rapidly rewire networks around the inhibited node. Negative feedback typically results in down-regulation of the signaling pathway, however inhibitors can result in re-activation of a pathway via relief of negative feedback. This has been seen in the PI3K-AKT-mTOR pathway where the mTORC1 inhibitor rapamycin caused increased AKT signaling in myeloma cells through loss of feedback from MTOR (125). Similarly, AKT inhibition results in pathway reactivation within hours, in this case via induced expression of receptor tyrosine kinases (RTKs) such as ERBB3 and IGF1R and INSR (126,127). The rapid transcriptional up-regulation of SGK1 (128,129) and/or SGK3 (130,131) in tumors suggests a central node of resistance in experimental models challenged with PI3K or AKT inhibitors. This is due to the ability of SGK family members, which encode similar substrate phosphorylation consensus specificity to AKT, to functionally replace this kinase in cells. The EGFR-RAS-RAF-MEK-ERK pathway is dysregulated in many cancers and inhibitors have been approved for many of these protein kinase nodes (Figure 4). However, the presence of feedback loops can lead to complex, and undesirable phenotypes in cells. Vemurafenib specifically inhibits the oncogenic V600E mutant form of BRAF (132). Rapid adaptation occurs within hours of vemurafenib treatment via loss of ERK (MAPK1/MAPK3)-dependent negative feedback that results in the restoration of RTK signaling and promotes the generation of inhibitor-resistant RAF dimers (133). A similar relief of feedback resistance mechanism that is dependent upon CRAF occurs in RAS mutant tumours when MEK (MAP2K1/MAP2K2) or ERK is inhibited (134). MEK inhibition also induces rapid rewiring of kinome networks via loss of ERK-dependent c-MYC expression resulting in increased expression of multiple RTKs and their ligands (90). Together, these responses are important for allowing the development of a sub-population of cells, sometimes called drug-resistant persistors (135) to survive the initial therapeutic assault before re-emerging and being reinforced by acquired resistance mechanisms.

Acquired resistance typically takes time to emerge via the acquisition of new mutations. The mechanism most classically apparent upon genome analysis is *via* mutations that interfere with inhibitor binding, typically inducing occlusion of the drug, loss of a favorable physiochemical interaction, or a change in an enzymatic property in the target. One of the first examples was revealed in the blood and bone marrow of CML patients treated with the ABL tyrosine kinase inhibitor imatinib (136,137). Importantly, BCR-ABL signaling was re-activated in patients that acquired a mutation resulting in a single amino acid substitution of a critical threonine residue in the ABL kinase domain (T315I) required for imatinib binding. Moreover, similar observations were made in experimental models of imatinib resistance, providing a convenient cell-based model for the evaluation of additional mutations (138) and establishing one of a suite of experimental cell and mass spectrometry-based approaches for the analysis of new allosteric BCR-ABL inhibitors developed to overcome drug-resistant CML (122,139). For the EGFR inhibitors gefitinib and erlotinib, resistance in lung cancer is associated with a T790M point mutation of the gatekeeper residue that markedly increases EGFR affinity for ATP, thereby competitively blocking the binding of type I EGFR inhibitors (140). To overcome this, irreversible (covalent) EGFR tyrosine kinase inhibitors such as osimertinib (and others in clinical development) were designed that are active against the mutated gatekeeper residue whilst exhibiting reduced potency towards wild type EGFR (141). However, resistance to these third generation EGFR inhibitors has already been documented, in some cases due to mutation of the critical covalent cysteine target (142,143). Alternative resistance mechanisms to EGFR therapies include genetic amplification of other RTKs such as MET (144), and the acquisition of activating mutations in downstream components that result in a bypass of the need for EGFR-mediated signaling (145).

An even greater variety of acquired resistance mechanisms have been characterized for BRAF and MEK inhibitors. In both cases, acquired resistance to these drugs results in

reactivation, that in most cases is due to RAF dimer mediated activation of ERK and indicates the critical dependence on MAP kinase signaling for tumor maintenance. Resistance mechanisms involving switching of signaling to parallel nodes (146,147), the emergence of gain-of-function mutations (148,149), *de novo* expression of other activators (150) and the emergence of activating mutations or amplifications of upstream and downstream components including BRAF, MEK and RTKs have all been identified in patients (151-157). Given the importance of ERK signaling and the rapid development of resistance within months of initiating treatment, new strategies involve more extensive personalized monitoring of biomarkers/genetic signatures of resistance to tailor therapy (158-160), the development of drugs that also limit oncogenic feedback mechanisms (161), and the concurrent targeting of multiple nodes within the same pathway to try to reduce the capacity of the system to survive via adaptive and acquired routes (162-165). Technological innovations in detecting cellular drug target binding, including the use of biophysical (166,167) and fluorescent drug tracking approaches (168) will increasingly be adopted to help evaluate target engagement and drug resistance. Finally, the existence and availability of curated, chemically diverse sets of cell permeable small molecules (169), perhaps most notably demonstrated by pioneering, open-access approaches to resource sharing to build a comprehensive kinase chemogenomic set (KCGS) (170-172) might permit small-scale research findings to be more rapidly translated into defined patient populations. Finally, and most crucially, the availability of collaborative datasets and validated chemical material firmly places control over drug-repurposing and refinement efforts for the human kinome within the reach of worldwide research communities.

DISCUSSION

We have presented a comprehensive overview of the human protein kinome highlighting the current state of knowledge, drug development and disease associations, and have made

this data freely available for each human kinase. It is striking that over half of the kinome remains very poorly understood despite this protein family being one of the most intensively studied over the last 50 years. Whilst generic features of kinase structure and biology can be extrapolated to many less well-studied kinase family members, the specific contributions of most kinases to cell biology and disease remain to be discovered. Similarly, the increasingly widespread use of phospho-proteomic analysis over the last 15 years has generated ~250,000 phospho-sites in human cells, yet <7% of these sites have a known kinase 'writer' and/or a known biological consequence for a phosphorylated protein substrate. This means that our ability to interpret complex datasets in the biological sense, and to understand information flow in kinase-regulated networks in order to develop mechanistic understanding is still at a preliminary stage. Endeavors in the next few years are likely to yield much more comprehensive information on regulatory phospho-sites, kinase-substrate relationships and the context-dependence of interactions. Effective assays of cellular kinome activity will also be necessary to more efficiently infer likely network activity from phospho-proteomic datasets. Proteogenomic data from technological drivers such as genomics, transcriptomics, mass spectrometry, chemical proteomics and high-level mapping of intracellular substrates and complexes needs to be more effective integrated so that more rapid traction can be made towards a whole kinome-level understanding of signaling.

Kinase dysregulation in disease is very well established and has been a major focus of biopharma efforts for decades. However, we are struck by the lack of concordance in major research reviews, articles and databases for assignment of a driver role to many individual kinases, even in intensively studied areas such as cancer. This likely reflects the significant context-dependence of kinase activity and illustrates the challenge for effective therapeutic intervention in individual patients. The excellent progress in developing kinase modulators for the clinic has significantly improved the outcomes for many patients. The new frontier in

finding effective drug combinations and dosing regimens for enhanced efficacy, whilst at the same time offsetting the emergence of resistance will benefit from the large number of -omic technologies and personalized treatment approaches that will be exploited over the coming years.

ACKNOWLEDGEMENTS

This work was supported by a BBSRC-AstraZeneca CASE studentship (BB/N504208/1), an MRC-AstraZeneca DiMeN CASE studentship, North West Cancer Research (NWCR) endowment and NWCR project grants (CR1037, CR1041 and CR1088). Competing interests: Paul Smith and Sarah Ross are employees and shareholders of AstraZeneca. The remaining authors declare that they have no competing interests.

REFEFERENCES

1. Krebs EG, Fischer EH. Phosphorylase activity of skeletal muscle extracts. J Biol Chem **1955**;216:113-20
2. Manning G, Whyte DB, Martinez R, Hunter T, Sudarsanam S. The protein kinase complement of the human genome. Science **2002**;298:1912-34
3. Hu Y, Furtmann N, Bajorath J. Current Compound Coverage of the Kinome. Journal of Medicinal Chemistry **2015**;58:30-40
4. Fedorov O, Muller S, Knapp S. The (un)targeted cancer kinome. Nat Chem Biol **2010**;6:166-9
5. Brognard J, Hunter T. Protein kinase signaling networks in cancer. Curr Opin Genet Dev **2011**;21:4-11
6. McSkimming DI, Dastgheib S, Baffi TR, Byrne DP, Ferries S, Scott ST*, et al.* KinView: a visual comparative sequence analysis tool for integrated kinome research. Mol Biosyst **2016**;12:3651-65
7. Sharma K, D'Souza RC, Tyanova S, Schaab C, Wisniewski JR, Cox J*, et al.* Ultradeep human phosphoproteome reveals a distinct regulatory nature of Tyr and Ser/Thr-based signaling. Cell Rep **2014**;8:1583-94
8. Chen MJ, Dixon JE, Manning G. Genomics and evolution of protein phosphatase genes. Science Signaling **2017**;10:eaag1796
9. Fabbro D. 25 years of small molecular weight kinase inhibitors: potentials and limitations. Mol Pharmacol **2015**;87:766-75
10. Gonzalez-Sanchez MB, Lanucara F, Helm M, Eyers CE. Attempting to rewrite History: challenges with the analysis of histidine-phosphorylated peptides. Biochem Soc Trans **2013**;41:1089-95
11. Fuhs SR, Meisenhelder J, Aslanian A, Ma L, Zagorska A, Stankova M*, et al.* Monoclonal 1- and 3-Phosphohistidine Antibodies: New Tools to Study Histidine Phosphorylation. Cell **2015**;162:198-210
12. Fuhs SR, Hunter T. pHisphorylation: the emergence of histidine phosphorylation as a reversible regulatory modification. Curr Opin Cell Biol **2017**;45:8-16
13. Baharani A, Trost B, Kusalik A, Napper S. Technological advances for interrogating the human kinome. Biochem Soc Trans **2017**;45:65-77
14. Mann M. Origins of mass spectrometry-based proteomics. Nat Rev Mol Cell Biol **2016**;17:678
15. Caron D, Byrne DP, Thebault P, Soulet D, Landry CR, Eyers PA*, et al.* Mitotic phosphotyrosine network analysis reveals that tyrosine phosphorylation regulates Polo-like kinase 1 (PLK1). Sci Signal **2016**;9:rs14
16. Kannan N, Taylor SS, Zhai Y, Venter JC, Manning G. Structural and functional diversity of the microbial kinome. PLoS Biol **2007**;5:e17
17. Wang T, Birsoy K, Hughes NW, Krupczak KM, Post Y, Wei JJ*, et al.* Identification and characterization of essential genes in the human genome. Science **2015**;350:1096-101
18. Hart T, Chandrashekhar M, Aregger M, Steinhart Z, Brown KR, MacLeod G*, et al.* High-Resolution CRISPR Screens Reveal Fitness Genes and Genotype-Specific Cancer Liabilities. Cell **2015**;163
19. Blomen VA, Majek P, Jae LT, Bigenzahn JW, Nieuwenhuis J, Staring J*, et al.* Gene essentiality and synthetic lethality in haploid human cells. Science **2015**;350:1092-6
20. Grueneberg DA, Degot S, Pearlberg J, Li W, Davies JE, Baldwin A*, et al.* Kinase requirements in human cells: I. Comparing kinase requirements across various cell types. Proc Natl Acad Sci U S A **2008**;105:16472-7

21. Deshmukh AS, Murgia M, Nagaraj N, Treebak JT, Cox J, Mann M. Deep Proteomics of Mouse Skeletal Muscle Enables Quantitation of Protein Isoforms, Metabolic Pathways, and Transcription Factors. Molecular & Cellular Proteomics **2015**;14:841-53
22. Kulak NA, Pichler G, Paron I, Nagaraj N, Mann M. Minimal, encapsulated proteomic-sample processing applied to copy-number estimation in eukaryotic cells. Nat Methods **2014**;11:319-24
23. Geiger T, Wehner A, Schaab C, Cox J, Mann M. Comparative Proteomic Analysis of Eleven Common Cell Lines Reveals Ubiquitous but Varying Expression of Most Proteins. Molecular & Cellular Proteomics **2012**;11
24. Gholami AM, Hahne H, Wu ZX, Auer FJ, Meng C, Wilhelm M*, et al.* Global Proteome Analysis of the NCI-60 Cell Line Panel. Cell Rep **2013**;4:609-20
25. Schwanhausser B, Busse D, Li N, Dittmar G, Schuchhardt J, Wolf J*, et al.* Global quantification of mammalian gene expression control. Nature **2012**;473:337-42
26. Pletscher-Frankild S, Palleja A, Tsafou K, Binder JX, Jensen LJ. DISEASES: Text mining and data integration of disease-gene associations. Methods **2015**;74:83-9
27. Blagg J, Workman P. Choose and use your chemical probe wisely to explore cancer biology. Cancer Cell **2017**;32:9-25
28. Taylor SS, Kornev AP. Protein kinases: evolution of dynamic regulatory proteins. Trends Biochem Sci **2011**;36:65-77
29. Johnson LN, Noble ME, Owen DJ. Active and inactive protein kinases: structural basis for regulation. Cell **1996**;85:149-58
30. Bayliss R, Fry A, Haq T, Yeoh S. On the molecular mechanisms of mitotic kinase activation. Open Biology **2012**;2
31. Eyers PA, Murphy JM. Dawn of the dead: protein pseudokinases signal new adventures in cell biology. Biochem Soc Trans **2013**;41:969-74
32. Reiterer V, Eyers PA, Farhan H. Day of the dead: pseudokinases and pseudophosphatases in physiology and disease. Trends Cell Biol **2014**;24:489-505
33. Murphy JM, Zhang Q, Young SN, Reese ML, Bailey FP, Eyers PA*, et al.* A robust methodology to subclassify pseudokinases based on their nucleotide-binding properties. Biochem J **2014**;457:323-34
34. Byrne DP, Foulkes DM, Eyers PA. Pseudokinases: update on their functions and evaluation as new drug targets. Future Med Chem **2017**;9:245-65
35. Sergina NV, Rausch M, Wang D, Blair J, Hann B, Shokat KM*, et al.* Escape from HER-family tyrosine kinase inhibitor therapy by the kinase-inactive HER3. Nature **2007**;445:437-41
36. Engelman JA, Zejnullahu K, Mitsudomi T, Song Y, Hyland C, Park JO*, et al.* MET amplification leads to gefitinib resistance in lung cancer by activating ERBB3 signaling. Science **2007**;316:1039-43
37. Shaw AS, Kornev AP, Hu J, Ahuja LG, Taylor SS. Kinases and pseudokinases: lessons from RAF. Mol Cell Biol **2014**;34:1538-46
38. Pinkas-Kramarski R, Soussan L, Waterman H, Levkowitz G, Alroy I, Klapper L*, et al.* Diversification of Neu differentiation factor and epidermal growth factor signaling by combinatorial receptor interactions. EMBO J **1996**;15:2452-67
39. Bailey FP, Byrne DP, McSkimming D, Kannan N, Eyers PA. Going for broke: targeting the human cancer pseudokinome. Biochem J **2015**;465:195-211
40. Otto T, Horn S, Brockmann M, Eilers U, Schuttrumpf L, Popov N*, et al.* Stabilization of N-Myc is a critical function of Aurora A in human neuroblastoma. Cancer Cell **2009**;15:67-78

41. Poulikakos PI, Zhang C, Bollag G, Shokat KM, Rosen N. RAF inhibitors transactivate RAF dimers and ERK signalling in cells with wild-type BRAF. Nature **2010**;464:427-30
42. Hall-Jackson CA, Eyers PA, Cohen P, Goedert M, Boyle FT, Hewitt N*, et al.* Paradoxical activation of Raf by a novel Raf inhibitor. Chem Biol **1999**;6:559-68
43. Wilhelm M, Schlegl J, Hahne H, Gholami AM, Lieberenz M, Savitski MM*, et al.* Mass-spectrometry-based draft of the human proteome. Nature **2014**;509:582-7
44. Hornbeck PV, Zhang B, Murray B, Kornhauser JM, Latham V, Skrzypek E. PhosphoSitePlus, 2014: mutations, PTMs and recalibrations. Nucleic Acids Research **2015**;43:D512-D20
45. Marx V. Making sure PTMs are not lost after translation. Nat Methods **2013**;10:201-4
46. Jersie-Christensen RR, Sultan A, Olsen JV. Simple and Reproducible Sample Preparation for Single-Shot Phosphoproteomics with High Sensitivity. Phospho-Proteomics: Methods and Protocols, 2nd Edition **2016**;1355:251-60
47. Leitner A. Enrichment Strategies in Phosphoproteomics. Phospho-Proteomics: Methods and Protocols, 2nd Edition **2016**;1355:105-21
48. Hunter T. Tyrosine-phosphorylation: thirty years and counting. Current Opinion in Cell Biology **2009**;21:140-6
49. Ha JR, Siegel PM, Ursini-Siegel J. The Tyrosine Kinome Dictates Breast Cancer Heterogeneity and Therapeutic Responsiveness. J Cell Biochem **2016**;117:1971-90
50. Gonzalez-Sanchez MB, Lanucara F, Hardman GE, Eyers CE. Gas-phase intermolecular phosphate transfer within a phosphohistidine phosphopeptide dimer. Int J Mass Spectrom **2014**;367:28-34
51. Olsen JV, Vermeulen M, Santamaria A, Kumar C, Miller ML, Jensen LJ*, et al.* Quantitative phosphoproteomics reveals widespread full phosphorylation site occupancy during mitosis. Sci Signal **2010**;3:ra3
52. Olsen JV, Blagoev B, Gnad F, Macek B, Kumar C, Mortensen P*, et al.* Global, in vivo, and site-specific phosphorylation dynamics in signaling networks. Cell **2006**;127:635-48
53. Tape CJ, Ling S, Dimitriadi M, McMahon KM, Worboys JD, Leong HS*, et al.* Oncogenic KRAS Regulates Tumor Cell Signaling via Stromal Reciprocation. Cell **2016**;165:910-20
54. Bondeson DP, Mares A, Smith IED, Ko E, Campos S, Miah AH*, et al.* Catalytic in vivo protein knockdown by small-molecule PROTACs. Nature Chemical Biology **2015**;11:611-U120
55. Tavassoli A. SICLOPPS cyclic peptide libraries in drug discovery. Curr Opin Chem Biol **2017**;38:30-5
56. Wang YX, Ho THG, Franz E, Hermann JS, Smith FD, Hehnly H*, et al.* PKA-Type I Selective Constrained Peptide Disruptors of AKAP Complexes. Acs Chem Biol **2015**;10:1502-10
57. Pinna LA, Ruzzene M. How do protein kinases recognize their substrates? Bba-Mol Cell Res **1996**;1314:191-225
58. Hu JF, Rho HS, Newman RH, Zhang J, Zhu H, Qian J. PhosphoNetworks: a database for human phosphorylation networks. Bioinformatics **2014**;30:141-2
59. Duarte ML, Pena DA, Ferraz FAN, Berti DA, Sobreira TJP, Costa HM*, et al.* Protein folding creates structure-based, noncontiguous consensus phosphorylation motifs recognized by kinases. Science Signaling **2014**;7
60. Hunter T. The age of crosstalk: phosphorylation, ubiquitination, and beyond. Mol Cell **2007**;28:730-8

61. Filipcik P, Curry JR, Mace PD. When Worlds Collide-Mechanisms at the Interface between Phosphorylation and Ubiquitination. J Mol Biol **2017**;429:1097-113
62. Hu JF, Rho HS, Newman RH, Hwang WC, Neiswinger J, Zhu H*, et al.* Global analysis of phosphorylation networks in humans. Bba-Proteins Proteom **2014**;1844:224-31
63. Huang KY, Wu HY, Chen YJ, Lu CT, Su MG, Hsieh YC*, et al.* RegPhos 2.0: an updated resource to explore protein kinase-substrate phosphorylation networks in mammals. Database-Oxford **2014**
64. Lee TY, Hsu JBK, Chang WC, Huang HD. RegPhos: a system to explore the protein kinase-substrate phosphorylation network in humans. Nucleic Acids Research **2011**;39:D777-D87
65. Newman RH, Hu JF, Rho HS, Xie Z, Woodard C, Neiswinger J*, et al.* Construction of human activity-based phosphorylation networks. Molecular Systems Biology **2013**;9
66. Yang PY, Humphrey SJ, James DE, Yang YH, Jothi R. Positive-unlabeled ensemble learning for kinase substrate prediction from dynamic phosphoproteomics data. Bioinformatics **2016**;32:252-9
67. Trost B, Kusalik A. Computational prediction of eukaryotic phosphorylation sites. Bioinformatics **2011**;27:2927-35
68. Xue Y, Liu ZX, Cao J, Ma QA, Gao XJ, Wang QQ*, et al.* GPS 2.1: enhanced prediction of kinase-specific phosphorylation sites with an algorithm of motif length selection. Protein Eng Des Sel **2011**;24:255-60
69. Horn H, Schoof EM, Kim J, Robin X, Miller ML, Diella F*, et al.* KinomeXplorer: an integrated platform for kinome biology studies. Nature Methods **2014**;11:603-4
70. Suo SB, Qiu JD, Shi SP, Chen X, Liang RP. PSEA: Kinase-specific prediction and analysis of human phosphorylation substrates. Sci Rep-Uk **2014**;4
71. Gao JJ, Thelen JJ, Dunker AK, Xu D. Musite, a Tool for Global Prediction of General and Kinase-specific Phosphorylation Sites. Molecular & Cellular Proteomics **2010**;9:2586-600
72. Zou L, Wang M, Shen Y, Liao J, Li A, Wang MH. PKIS: computational identification of protein kinases for experimentally discovered protein phosphorylation sites. Bmc Bioinformatics **2013**;14
73. de Oliveira PSL, Ferraz FAN, Pena DA, Pramio DT, Morais FA, Schechtman D. Revisiting protein kinase-substrate interactions: Toward therapeutic development. Science Signaling **2016**;9
74. Linding R, Jensen LJ, Pasculescu A, Olhovsky M, Colwill K, Bork P*, et al.* NetworKIN: a resource for exploring cellular phosphorylation networks. Nucleic Acids Res **2008**;36:D695-9
75. Ellis JJ, Kobe B. Predicting Protein Kinase Specificity: Predikin Update and Performance in the DREAM4 Challenge. Plos One **2011**;6
76. Gnad F, Ren SB, Cox J, Olsen JV, Macek B, Oroshi M*, et al.* PHOSIDA (phosphorylation site database): management, structural and evolutionary investigation, and prediction of phosphosites. Genome Biol **2007**;8
77. Patrick R, Le Cao KA, Kobe B, Boden M. PhosphoPICK: modelling cellular context to map kinase-substrate phosphorylation events. Bioinformatics **2015**;31:382-9
78. Reddy RJ, Gajadhar AS, Swenson EJ, Rothenberg DA, Curran TG, White FM. Early signaling dynamics of the epidermal growth factor receptor. Proc Natl Acad Sci U S A **2016**;113:3114-9
79. Bain J, Plater L, Elliott M, Shpiro N, Hastie CJ, Mclauchlan H*, et al.* The selectivity of protein kinase inhibitors: a further update. Biochemical Journal **2007**;408:297-315

80. Worboys JD, Sinclair J, Yuan YY, Jorgensen C. Systematic evaluation of quantotypic peptides for targeted analysis of the human kinome. Nature Methods **2014**;11:1041-4
81. Zhang LX, Holmes IP, Hochgrafe F, Walker SR, Ali NA, Humphrey ES*, et al.* Characterization of the Novel Broad-Spectrum Kinase Inhibitor CTx-0294885 As an Affinity Reagent for Mass Spectrometry-Based Kinome Profiling. Journal of Proteome Research **2013**;12:3104-16
82. Medard G, Pachl F, Ruprecht B, Klaeger S, Heinzlmeir S, Helm D*, et al.* Optimized Chemical Proteomics Assay for Kinase Inhibitor Profiling. Journal of Proteome Research **2015**;14:1574-86
83. Lemeer S, Zorgiebel G, Ruprecht B, Kohl K, Kuster B. Comparing Immobilized Kinase Inhibitors and Covalent ATP Probes for Proteomic Profiling of Kinase Expression and Drug Selectivity. Journal of Proteome Research **2013**;12:1723-31
84. Oppermann FS, Gnad F, Olsen JV, Hornberger R, Greff Z, Keri G*, et al.* Large-scale Proteomics Analysis of the Human Kinome. Molecular & Cellular Proteomics **2009**;8:1751-64
85. Daub H, Olsen JV, Bairlein M, Gnad F, Oppermann FS, Korner R*, et al.* Kinase-selective enrichment enables quantitative phosphoproteomics of the kinome across the cell cycle. Molecular Cell **2008**;31:438-48
86. Bantscheff M, Eberhard D, Abraham Y, Bastuck S, Boesche M, Hobson S*, et al.* Quantitative chemical proteomics reveals mechanisms of action of clinical ABL kinase inhibitors. Nature Biotechnology **2007**;25:1035-44
87. Stuhlmiller TJ, Miller SM, Zawistowski JS, Nakamura K, Beltran AS, Duncan JS*, et al.* Inhibition of Lapatinib-Induced Kinome Reprogramming in ERBB2-Positive Breast Cancer by Targeting BET Family Bromodomains. Cell Rep **2015**;11:390-404
88. Zawistowski JS, Graves LM, Johnson GL. Assessing adaptation of the cancer kinome in response to targeted therapies. Biochem Soc T **2014**;42:765-9
89. Cooper MJ, Cox NJ, Zimmerman EI, Dewar BJ, Duncan JS, Whittle MC*, et al.* Application of Multiplexed Kinase Inhibitor Beads to Study Kinome Adaptations in Drug-Resistant Leukemia. Plos One **2013**;8
90. Duncan JS, Whittle MC, Nakamura K, Abell AN, Midland AA, Zawistowski JS*, et al.* Dynamic reprogramming of the kinome in response to targeted MEK inhibition in triple-negative breast cancer. Cell **2012**;149:307-21
91. Ruprecht B, Zecha J, Heinzlmeir S, Medard G, Lemeer S, Kuster B. Evaluation of Kinase Activity Profiling Using Chemical Proteomics. Acs Chem Biol **2015**;10:2743-52
92. Boja ES, Rodriguez H. Proteogenomic convergence for understanding cancer pathways and networks. Clin Proteom **2014**;11
93. Nesvizhskii AI. Proteogenomics: concepts, applications and computational strategies. Nature Methods **2014**;11:1114-25
94. Olow A, Chen Z, Niedner RH, Wolf DM, Yau C, Pankov A*, et al.* An Atlas of the Human Kinome Reveals the Mutational Landscape Underlying Dysregulated Phosphorylation Cascades in Cancer. Cancer Res **2016**;76:1733-45
95. Singleton AB, Farrer MJ, Bonifati V. The Genetics of Parkinson's Disease: Progress and Therapeutic Implications. Movement Disord **2013**;28:14-23
96. Duchon A, Herault Y. DYRK1A, a Dosage-Sensitive Gene Involved in Neurodevelopmental Disorders, Is a Target for Drug Development in Down Syndrome. Front Behav Neurosci **2016**;10
97. Gerull B. The Rapidly Evolving Role of Titin in Cardiac Physiology and Cardiomyopathy. Can J Cardiol **2015**;31:1351-9

98. Simpson MA, Hsu R, Keir LS, Hao J, Sivapalan G, Ernst LM*, et al.* Mutations in FAM20C are associated with lethal osteosclerotic bone dysplasia (Raine syndrome), highlighting a crucial molecule in bone development. Am J Hum Genet **2007**;81:906-12
99. Futreal PA, Coin L, Marshall M, Down T, Hubbard T, Wooster R*, et al.* A census of human cancer genes. Nature Reviews Cancer **2004**;4:177-83
100. Lawrence MS, Stojanov P, Polak P, Kryukov GV, Cibulskis K, Sivachenko A*, et al.* Mutational heterogeneity in cancer and the search for new cancer-associated genes. Nature **2013**;499:214-8
101. Fleuren EDG, Zhang LX, Wu JM, Daly RJ. The kinome 'at large' in cancer. Nature Reviews Cancer **2016**;16:83-98
102. Pearson A, Smyth E, Babina IS, Herrera-Abreu MT, Tarazona N, Peckitt C*, et al.* High-Level Clonal FGFR Amplification and Response to FGFR Inhibition in a Translational Clinical Trial. Cancer Discovery **2016**;6:838-51
103. Slamon DJ, Clark GM, Wong SG, Levin WJ, Ullrich A, Mcguire WL. Human-Breast Cancer - Correlation of Relapse and Survival with Amplification of the Her-2 Neu Oncogene. Science **1987**;235:177-82
104. Testa U, Pelosi E. The Impact of FLT3 Mutations on the Development of Acute Myeloid Leukemias. Leuk Res Treatment **2013**;2013:275760
105. Antal CE, Hudson AM, Kang E, Zanca C, Wirth C, Stephenson NL*, et al.* Cancer-associated protein kinase C mutations reveal kinase's role as tumor suppressor. Cell **2015**;160:489-502
106. Nieto P, Ambrogio C, Esteban-Burgos L, Gomez-Lopez G, Blasco MT, Yao Z*, et al.* A Braf kinase-inactive mutant induces lung adenocarcinoma. Nature **2017**;548:239-43
107. Gaulton A, Hersey A, Nowotka M, Bento AP, Chambers J, Mendez D*, et al.* The ChEMBL database in 2017. Nucleic Acids Res **2017**;45:D945-D54
108. Santos R, Ursu O, Gaulton A, Bento AP, Donadi RS, Bologa CG*, et al.* A comprehensive map of molecular drug targets. Nature Reviews in Drug Discovery **2017**;16:19-34
109. Tym JE, Mitsopoulos C, Coker EA, Razaz P, Schierz AC, Antolin AA*, et al.* canSAR: an updated cancer research and drug discovery knowledgebase. Nucleic Acids Research **2016**;44:D938-D43
110. Gaubitz C, Oliveira TM, Prouteau M, Leitner A, Karuppasamy M, Konstantinidou G*, et al.* Molecular Basis of the Rapamycin Insensitivity of Target Of Rapamycin Complex 2. Mol Cell **2015**;58:977-88
111. Roskoski R, Jr. Janus kinase (JAK) inhibitors in the treatment of inflammatory and neoplastic diseases. Pharmacol Res **2016**;111:784-803
112. Harbeck N, Beckmann MW, Rody A, Schneeweiss A, Muller V, Fehm T*, et al.* HER2 Dimerization Inhibitor Pertuzumab - Mode of Action and Clinical Data in Breast Cancer. Breast Care **2013**;8:49-55
113. Koide A, Abbatiello S, Rothgery L, Koide S. Probing protein conformational changes in living cells by using designer binding proteins: application to the estrogen receptor. Proc Natl Acad Sci U S A **2002**;99:1253-8
114. Brierley SM. Guanylate cyclase-C receptor activation: unexpected biology. Curr Opin Pharmacol **2012**;12:632-40
115. Shibuya M. Vascular endothelial growth factor and its receptor system: physiological functions in angiogenesis and pathological roles in various diseases. J Biochem **2013**;153:13-9

116. Ciceri P, Muller S, O'Mahony A, Fedorov O, Filippakopoulos P, Hunt JP*, et al.* Dual kinase-bromodomain inhibitors for rationally designed polypharmacology. Nat Chem Biol **2014**;10:305-12
117. Klaeger S, Gohlke B, Perrin J, Gupta V, Heinzlmeir S, Helm D*, et al.* Chemical Proteomics Reveals Ferrochelatase as a Common Off-target of Kinase Inhibitors. ACS Chem Biol **2016**;11:1245-54
118. Haystead TA. The purinome, a complex mix of drug and toxicity targets. Curr Top Med Chem **2006**;6:1117-27
119. Davis MI, Hunt JP, Herrgard S, Ciceri P, Wodicka LM, Pallares G*, et al.* Comprehensive analysis of kinase inhibitor selectivity. Nat Biotechnol **2011**;29:1046-51
120. Wu P, Clausen MH, Nielsen TE. Allosteric small-molecule kinase inhibitors. Pharmacol Therapeut **2015**;156:59-68
121. Caunt CJ, Sale MJ, Smith PD, Cook SJ. MEK1 and MEK2 inhibitors and cancer therapy: the long and winding road. Nature Reviews Cancer **2015**;15:577-92
122. Wylie AA, Schoepfer J, Jahnke W, Cowan-Jacob SW, Loo A, Furet P*, et al.* The allosteric inhibitor ABL001 enables dual targeting of BCR-ABL1. Nature **2017**;543:733-737.
123. Levitzki A. Tyrosine Kinase Inhibitors: Views of Selectivity, Sensitivity, and Clinical Performance. Annu Rev Pharmacol **2013**;53:161-85
124. Orphanos GS, Ioannidis GN, Ardavanis AG. Cardiotoxicity induced by tyrosine kinase inhibitors. Acta Oncol **2009**;48:964-70
125. Shi YJ, Yan HJ, Frost P, Gera J, Lichtenstein A. Mammalian target of rapamycin inhibitors activate the AKT kinase in multiple myeloma cells by up-regulating the insulin-like growth factor receptor/insulin receptor substrate-1/phosphatidylinositol 3-kinase cascade. Molecular Cancer Therapeutics **2005**;4:1533-40
126. Chandarlapaty S, Sawai A, Scaltriti M, Rodrik-Outmezguine V, Grbovic-Huezo O, Serra V*, et al.* AKT Inhibition Relieves Feedback Suppression of Receptor Tyrosine Kinase Expression and Activity. Cancer Cell **2011**;19:58-71
127. Crafter C, Vincent JP, Tang E, Dudley P, James NH, Klinowska T*, et al.* Combining AZD8931, a novel EGFR/HER2/HER3 signalling inhibitor, with AZD5363 limits AKT inhibitor induced feedback and enhances antitumour efficacy in HER2-amplified breast cancer models. Int J Oncol **2015**;47:446-54
128. Castel P, Ellis H, Bago R, Toska E, Razavi P, Carmona FJ*, et al.* PDK1-SGK1 Signaling Sustains AKT-Independent mTORC1 Activation and Confers Resistance to PI3K alpha Inhibition. Cancer Cell **2016**;30:229-42
129. Sommer EM, Dry H, Cross D, Guichard S, Davies BR, Alessi DR. Elevated SGK1 predicts resistance of breast cancer cells to Akt inhibitors. Biochemical Journal **2013**;452:499-508
130. Vasudevan KM, Barbie DA, Davies MA, Rabinovsky R, McNear CJ, Kim JJ*, et al.* AKT-independent signaling downstream of oncogenic PIK3CA mutations in human cancer. Cancer Cell **2009**;16:21-32
131. Bago R, Sommer E, Castel P, Crafter C, Bailey FP, Shpiro N*, et al.* The hVps34-SGK3 pathway alleviates sustained PI3K/Akt inhibition by stimulating mTORC1 and tumour growth. Embo J **2016**;35:1902-22
132. Tsai J, Lee JT, Wang W, Zhang J, Cho H, Mamo S*, et al.* Discovery of a selective inhibitor of oncogenic B-Raf kinase with potent antimelanoma activity. P Natl Acad Sci USA **2008**;105:3041-6

133. Lito P, Pratilas CA, Joseph EW, Tadi M, Halilovic E, Zubrowski M*, et al.* Relief of Profound Feedback Inhibition of Mitogenic Signaling by RAF Inhibitors Attenuates Their Activity in BRAFV600E Melanomas. Cancer Cell **2012**;22:668-82
134. Lito P, Saborowski A, Yue JY, Solomon M, Joseph E, Gadal S*, et al.* Disruption of CRAF-Mediated MEK Activation Is Required for Effective MEK Inhibition in KRAS Mutant Tumors. Cancer Cell **2014**;25:697-710
135. Ramirez M, Rajaram S, Steininger RJ, Osipchuk D, Roth MA, Morinishi LS*, et al.* Diverse drug-resistance mechanisms can emerge from drug-tolerant cancer persister cells. Nat Commun **2016**;7:10690
136. Shah NP, Nicoll JM, Nagar B, Gorre ME, Paquette RL, Kuriyan J*, et al.* Multiple BCR-ABL kinase domain mutations confer polyclonal resistance to the tyrosine kinase inhibitor imatinib (STI571) in chronic phase and blast crisis chronic myeloid leukemia. Cancer Cell **2002**;2:117-25
137. Gorre ME, Mohammed M, Ellwood K, Hsu N, Paquette R, Rao PN*, et al.* Clinical resistance to STI-571 cancer therapy caused by BCR-ABL gene mutation or amplification. Science **2001**;293:876-80
138. Azam M, Latek RR, Daley GQ. Mechanisms of autoinhibition and STI-571/imatinib resistance revealed by mutagenesis of BCR-ABL. Cell **2003**;112:831-43
139. Parker WT, Yeung DT, Yeoman AL, Altamura HK, Jamison BA, Field CR*, et al.* The impact of multiple low-level BCR-ABL1 mutations on response to ponatinib. Blood **2016**;127:1870-80
140. Pao W, Miller VA, Politi KA, Riely GJ, Somwar R, Zakowski MF*, et al.* Acquired resistance of lung adenocarcinomas to gefitinib or erlotinib is associated with a second mutation in the EGFR kinase domain. Plos Med **2005**;2:225-35
141. Wang SH, Cang SD, Liu DL. Third-generation inhibitors targeting EGFR T790M mutation in advanced non-small cell lung cancer. J Hematol Oncol **2016**;9
142. Niederst MJ, Hu HC, Mulvey HE, Lockerman EL, Garcia AR, Piotrowska Z*, et al.* The Allelic Context of the C797S Mutation Acquired upon Treatment with Third-Generation EGFR Inhibitors Impacts Sensitivity to Subsequent Treatment Strategies. Clinical Cancer Research **2015**;21:3924-33
143. Thress KS, Paweletz CP, Felip E, Cho BC, Stetsonl D, Dougherty B*, et al.* Acquired EGFR C797S mutation mediates resistance to AZD9291 in non-small cell lung cancer harboring EGFR T790M. Nat Med **2015**;21:560-2
144. Bardelli A, Corso S, Bertotti A, Hobor S, Valtorta E, Siravegna G*, et al.* Amplification of the MET Receptor Drives Resistance to Anti-EGFR Therapies in Colorectal Cancer. Cancer Discovery **2013**;3:658-73
145. Sequist LV, Waltman BA, Dias-Santagata D, Digumarthy S, Turke AB, Fidias P*, et al.* Genotypic and Histological Evolution of Lung Cancers Acquiring Resistance to EGFR Inhibitors. Sci Transl Med **2011**;3
146. Montagut C, Sharma SV, Shioda T, McDermott U, Ulman M, Ulkus LE*, et al.* Elevated CRAF as a potential mechanism of acquired resistance to BRAF inhibition in melanoma. Cancer Res **2008**;68:4853-61
147. Villanueva J, Vultur A, Lee JT, Somasundaram R, Fukunaga-Kalabis M, Cipolla AK*, et al.* Acquired Resistance to BRAF Inhibitors Mediated by a RAF Kinase Switch in Melanoma Can Be Overcome by Cotargeting MEK and IGF-1R/PI3K. Cancer Cell **2010**;18:683-95
148. Poulikakos PI, Persaud Y, Janakiraman M, Kong XJ, Ng C, Moriceau G*, et al.* RAF inhibitor resistance is mediated by dimerization of aberrantly spliced BRAF(V600E). Nature **2011**;480:387-U144

149. Emery CM, Vijayendran KG, Zipser MC, Sawyer AM, Niu LL, Kim JJ*, et al.* MEK1 mutations confer resistance to MEK and B-RAF inhibition. P Natl Acad Sci USA **2009**;106:20411-6
150. Johannessen CM, Boehm JS, Kim SY, Thomas SR, Wardwell L, Johnson LA*, et al.* COT drives resistance to RAF inhibition through MAP kinase pathway reactivation. Nature **2010**;468:968-U370
151. Nazarian R, Shi HB, Wang Q, Kong XJ, Koya RC, Lee H*, et al.* Melanomas acquire resistance toB-RAF(V600E) inhibition by RTK or N-RAS upregulation. Nature **2010**;468:973-U377
152. Shi HB, Moriceau G, Kong XJ, Lee MK, Lee H, Koya RC*, et al.* Melanoma whole-exome sequencing identifies B-V600E-RAF amplification-mediated acquired B-RAF inhibitor resistance. Nat Commun **2012**;3
153. Yadav V, Zhang XY, Liu JG, Estrem S, Li SY, Gong XQ*, et al.* Reactivation of Mitogen-activated Protein Kinase (MAPK) Pathway by FGF Receptor 3 (FGFR3)/Ras Mediates Resistance to Vemurafenib in Human B-RAF V600E Mutant Melanoma. Journal of Biological Chemistry **2012**;287:28087-98
154. Villanueva J, Infante JR, Krepler C, Reyes-Uribe P, Samanta M, Chen HY*, et al.* Concurrent MEK2 Mutation and BRAF Amplification Confer Resistance to BRAF and MEK Inhibitors in Melanoma. Cell Rep **2013**;4:1090-9
155. Little AS, Balmanno K, Sale MJ, Newman S, Dry JR, Hampson M*, et al.* Amplification of the Driving Oncogene, KRAS or BRAF, Underpins Acquired Resistance to MEK1/2 Inhibitors in Colorectal Cancer Cells (vol 4, art no ra17, 2011). Science Signaling **2011**;4
156. Corcoran RB, Dias-Santagata D, Bergethon K, Iafrate AJ, Settleman J, Engelman JA. BRAF Gene Amplification Can Promote Acquired Resistance to MEK Inhibitors in Cancer Cells Harboring the BRAF V600E Mutation. Science Signaling **2010**;3
157. Su F, Viros A, Milagre C, Trunzer K, Bollag G, Spleiss O*, et al.* RAS Mutations in Cutaneous Squamous-Cell Carcinomas in Patients Treated with BRAF Inhibitors. New Engl J Med **2012**;366:207-15
158. Brant R, Sharpe A, Liptrot T, Dry JR, Harrington EA, Barrett JC*, et al.* Clinically Viable Gene Expression Assays with Potential for Predicting Benefit from MEK Inhibitors. Clin Cancer Res **2017**;23:1471-80
159. Dry JR, Pavey S, Pratilas CA, Harbron C, Runswick S, Hodgson D*, et al.* Transcriptional Pathway Signatures Predict MEK Addiction and Response to Selumetinib (AZD6244). Cancer Res **2010**;70:2264-73
160. Krepler C, Xiao M, Sproesser K, Brafford PA, Shannan B, Beqiri M*, et al.* Personalized Preclinical Trials in BRAF Inhibitor-Resistant Patient-Derived Xenograft Models Identify Second-Line Combination Therapies. Clin Cancer Res **2016**;22:1592-602
161. Ishii N, Harada N, Joseph EW, Ohara K, Miura T, Sakamoto H*, et al.* Enhanced Inhibition of ERK Signaling by a Novel Allosteric MEK Inhibitor, CH5126766, That Suppresses Feedback Reactivation of RAF Activity. Cancer Res **2013**;73:4050-60
162. Lamba S, Russo M, Sun C, Lazzari L, Cancelliere C, Grernrum W*, et al.* RAF Suppression Synergizes with MEK Inhibition in KRAS Mutant Cancer Cells. Cell Rep **2014**;8:1475-83
163. Long GV, Stroyakovskiy D, Gogas H, Levchenko E, de Braud F, Larkin J*, et al.* Combined BRAF and MEK Inhibition versus BRAF Inhibition Alone in Melanoma. New Engl J Med **2014**;371:1877-88

164. Flaherty KT, Infante JR, Daud A, Gonzalez R, Kefford RF, Sosman J*, et al.* Combined BRAF and MEK Inhibition in Melanoma with BRAF V600 Mutations. New Engl J Med **2012**;367:1694-703
165. Korkut A, Wang WQ, Demir E, Aksoy BA, Jing XH, Molinelli EJ*, et al.* Perturbation biology nominates upstream-downstream drug combinations in RAF inhibitor resistant melanoma cells. Elife **2015**;4
166. Savitski MM, Reinhard FB, Franken H, Werner T, Savitski MF, Eberhard D*, et al.* Tracking cancer drugs in living cells by thermal profiling of the proteome. Science **2014**;346:1255784
167. Martinez Molina D, Jafari R, Ignatushchenko M, Seki T, Larsson EA, Dan C*, et al.* Monitoring drug target engagement in cells and tissues using the cellular thermal shift assay. Science **2013**;341:84-7
168. Robers MB, Dart ML, Woodroofe CC, Zimprich CA, Kirkland TA, Machleidt T*, et al.* Target engagement and drug residence time can be observed in living cells with BRET. Nat Commun **2015**;6:10091
169. Arrowsmith CH, Audia JE, Austin C, Baell J, Bennett J, Blagg J*, et al.* The promise and peril of chemical probes. Nat Chem Biol **2015**;11:536-41
170. Elkins JM, Fedele V, Szklarz M, Abdul Azeez KR, Salah E, Mikolajczyk J*, et al.* Comprehensive characterization of the Published Kinase Inhibitor Set. Nat Biotechnol **2016**;34:95-103
171. Billin AN, Bantscheff M, Drewes G, Ghidelli-Disse S, Holt JA, Kramer HF*, et al.* Discovery of Novel Small Molecules that Activate Satellite Cell Proliferation and Enhance Repair of Damaged Muscle. ACS Chem Biol **2016**;11:518-29
172. Drewry DH, Wells CI, Andrews DM, Angell R, Al-Ali H, Axtman AD*, et al.* Progress towards a public chemogenomic set for protein kinases and a call for contributions. PLoS One **2017**;12:e0181585

Kinome ontology/overview	
KinBASE	http://www.kinase.com/
KinaseNET	http://www.kinasenet.ca/
ProKinO	http://vulcan.cs.uga.edu/prokino
Phosphorylation/kinase-substrate resources	
GPS 3.0	http://gps.biocuckoo.org/
HPRD	http://www.hprd.org/
Phospho.ELM	http://phospho.elm.eu.org/
PHOSIDA	http://141.61.102.18/phosida/index.aspx
PhoshoNET	http://www.phosphonet.ca/
PhosphoNetworks	http://www.phosphonetworks.org/
PhosphoSitePlus	https://www.phosphosite.org
RegPhos 2.0	http://140.138.144.141/~RegPhos/index.php
Kinase drug sensitivity and affinity measurements	
canSAR 3.0	http://cansar.icr.ac.uk
ChEMBL	https://www.ebi.ac.uk/chembldb/
Drugbank	https://www.drugbank.ca/
DrugKiNET	http://www.drugkinet.ca/
Kinase SARfari	https://www.ebi.ac.uk/chembl/sarfari/kinasesarfari
International Centre for Kinase Profiling	http://www.kinase-screen.mrc.ac.uk/
Disease associations	
COSMIC	http://cancer.sanger.ac.uk/cosmic
DECIPHER	http://diseases.jensenlab.org
DISEASES	https://decipher.sanger.ac.uk/
TCGA	https://cancergenome.nih.gov/
UniProtKB	http://www.uniprot.org/uniprot/

Table 1. *Kinase databases and resources*
Links to currently available web resources relevant to kinome and kinase biology.

FIGURE LEGENDS

Figure 1. *An overview of the protein kinome knowledge base.*

The activity, cellular requirement, disease association, availability of protein structures and drugs and research publications associated with each of the 535 members of the human protein kinome are displayed. Details and citations for sources of data are described in Supplementary Table 1.

Figure 2. *Phosphorylation and kinase consensus motifs*

Almost 250,000 phospho-sites have been experimentally detected within the human proteome although less than 3% have a known functional effect on the target protein (data curated from www.phosphosite.org; (44)). Curation of 301 experimentally determined kinases consensus motifs (Supplementary Table 2; (57,58)) highlight the differences in adjacent charged, bulky and hydrophobic residue requirements for tyrosine versus serine/threonine phosphorylation. Motifs are indicated if they represent ≥30% of the types of amino acids observed in that position on the substrates phosphorylated by the indicated kinase. Amino acids are indicated if they were specifically observed ≥20% of the time (small letter) or ≥50% of the time (large letter).

Figure 3. *Mutation and copy number alteration frequencies of kinases in selected human cancers*

Tyrosine kinases and Tyrosine kinase like (TKL) family members disproportionately exhibit copy number alterations (CNA) representing amplifications (dark blue) or deletions (light blue) and/or significant mutation rates (red) compared to other major kinome families. Circle size indicates the % of patients exhibiting CNA or mutations. Values at the bottom of the table indicate the number of kinases showing ≥5% CNA and/or significant mutation rates

(MutSigCV v0.9, q≤0.1; False Discovery Rate, Benjamini-Hochberg procedure (100)) in at least one TCGA cancer type. The bubble plot highlights a subset of cancer relevant kinases including many of the most mutated and/or copy number altered kinases within the kinome. The results here are in part based upon data generated by the TCGA Research Network: http://cancergenome.nih.gov/ and available via http://firebrowse.org/.

Figure 4. *FDA approved kinome therapeutics*

Generic names of kinase inhibitors or modulators, the year of FDA approval for therapeutic use and their major kinase targets are described. The majority of chemical inhibitors are thought to be relatively promiscuous at therapeutic doses, permitting the same drugs to be used in distinct kinase-driven disease indications, but enhancing the risk of 'off-target' effects such as kinase (or non-kinase)-associated cell cytotoxicity.

Figure 5. *Kinome responses to FDA approved chemical inhibitors of kinases*

The selectivity and potency of approved kinase inhibitors is highly variable. The IC_{50} and K_D values indicated represent the lowest experimentally determined value that is publicly available and are biased towards well-studied canonical kinases. No single protocol, enzyme source or substrate has been used to generate these values and therefore they should be only used as an approximation of potency or selectivity, especially as the set concentration of competing ATP is usually orders of magnitude lower than that found in human cells. See Supplementary Table 2 for data. Kinome drug sensitivities were plotted using TREE*spot* v5.0 and reprinted with permission from KINOME*scan*®, a division of DiscoveRx Corporation.

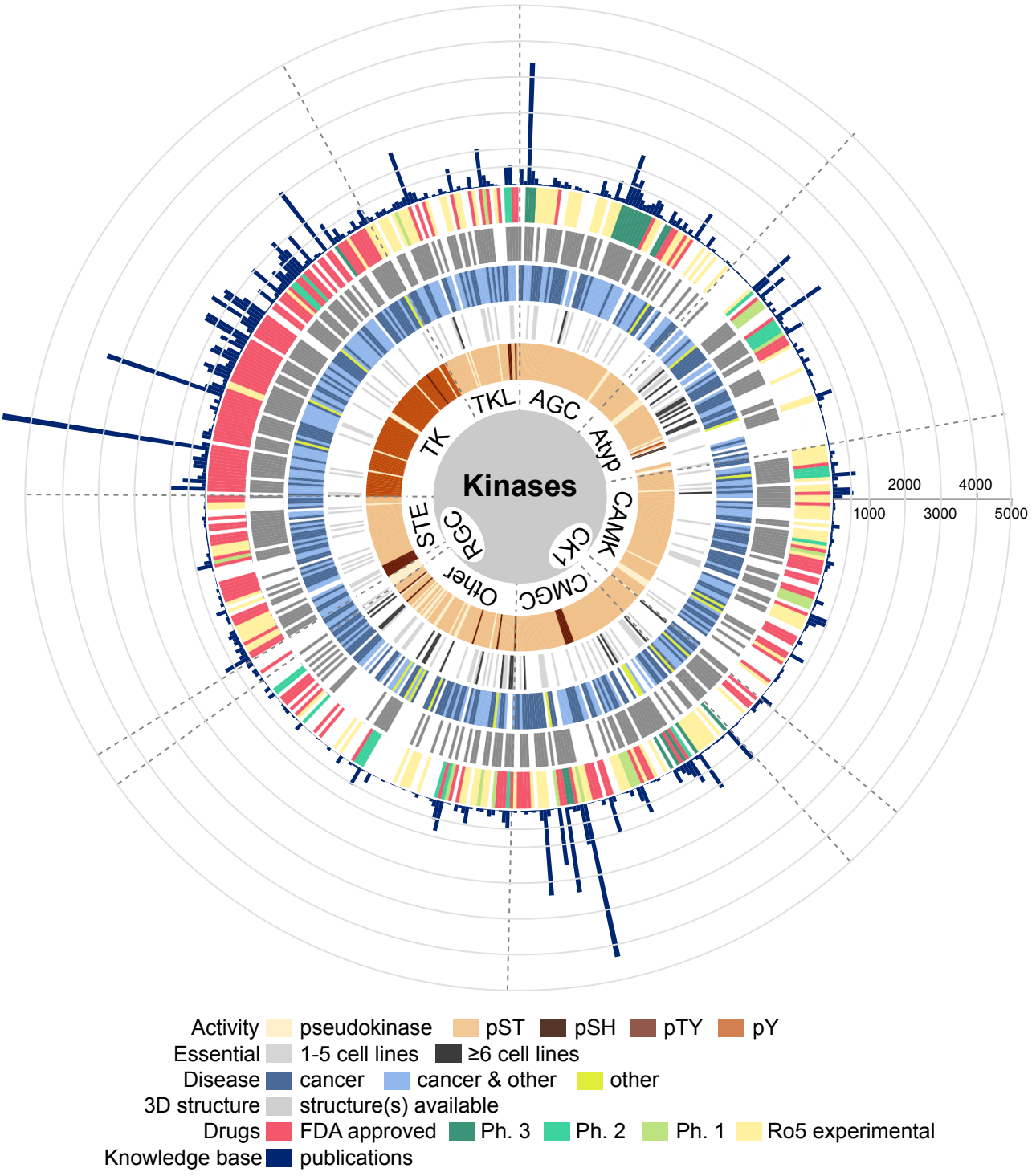
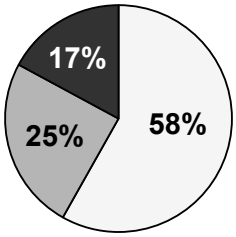

Kinases
TKL
AGC
Atyp
CAMK
CK1
CMGC
Other
RGC
STE
TK
1000
2000
3000
4000
5000
Activity pseudokinase pST pSH pTY pY
Essential 1-5 cell lines ≥6 cell lines
Disease cancer cancer & other other
3D structure structure(s) available
Drugs FDA approved Ph. 3 Ph. 2 Ph. 1 Ro5 experimental
Knowledge base publications

Figure 1

Phosphosite census

Human phosphosites

total

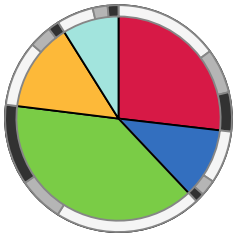


223,902 sites

pSer pThr pTyr

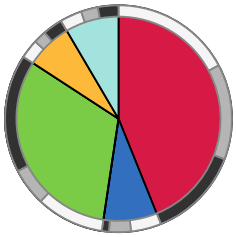
Regulatory phosphosites

total



5,330 sites

Kinase phosphosites



993 sites

altered activity
altered localisation
protein interactions
protein stability
other

Kinase consensus motifs

Gene name	Group	-7	-6	-5	-4	-3	-2	-1	0	1	2	3	4	5	6	7
AKT1	AGC	•	•	R	•	R	S	•	ST		•	•	•	•	S	•
PDPK1	AGC	•	•	•	•	•	•	•	ST		•	G	T	P	•	
PRKACA	AGC	•	•	•	•	R	R	•	ST		•	•	•	•	•	•
PRKCA	AGC	•	•	•	•	R	R	•	ST		RK	•	•	•	•	•
PRKCD	AGC	•	•	•	•	R	R	•	ST			•	•	•	•	•
PRKG1	AGC		•		•	R	RK		ST	•	•	•	•	•	•	L
CAMK2A	CAMK	•	•		•	R	•	•	ST			•		•	•	
CHEK2	CAMK	PS	•	L	•	R	•	•	ST	•	•	•	•	•	•	•
PIM1	CAMK	•	•	R		R	•	•	ST	•	S	•	•	•	•	•
PRKAA1	CAMK	•	•		•	R	•	•	ST	S	•	•		•	•	
STK11	CAMK	T	•	G	•		L	•	ST	•	•	G	S	P	P	Y
CSNK1D	CK1	S	•	•	S	S	•	•	ST	P	S	S	•	•	S	•
CDK1	CMGC	•	•	•	•		P	•	ST	P	•	K	•	•	•	•
CDK5	CMGC	•	•	•	S	•	P	•	ST	P	•		S	•	•	•
GSK3B	CMGC	•	•	•	S	•	•	•	ST	P	P	•	S	P	•	•
MAPK1	CMGC	•	•	•	•	•	P	•	ST	P	•	•	•	•	•	•
AURKB	Other	•	•	•	•	R	R		ST	•	•	•	•	•	•	•
IKBKB	Other	•	•	•			L	•	ST		•	•	S	•		•
PLK1	Other	•	•	•		•	DE		ST			•	•	•	•	S
WEE1	Other	•	P	P	•	R	S	P	ST	P	•	•	•	•	•	•
MAP2K2	STE	•	S	•	•	•		L	ST	P		•	•	T		•
PAK1	STE	•	•	•	•	R	R	•	ST		•	G	•	•	•	•
RAF1	TKL	•	•	R	•	R	S	•	ST	•	•	S	•	•	•	•
RIPK2	TKL	R	G	R	S	R	S	R	ST	•	S	•	S	•	•	G
TGFBR2	TKL	•	•	•	•	•	•	G	ST	G	S	•	•	•	•	
WEE1	Other	•		•	G	•	G	•	Y	G	•		•	•	•	
MAP2K2	STE	R	E	•	F	•	P	•	Y	T	A			•	Y	R
ABL2	TK	•	G		E		•	•	Y	•	•	P	V	G	•	•
BTK	TK	E		•	S	•	•	L	Y	D	S	•	•	•		•
CSK	TK	F	T	SA	T	E	P	•	Y	•	P	G	E	•	L	IS
EGFR	TK	•	•	•	E			•	Y		•		•	•	•	•
FGFR2	TK	•	G	P	•	•	P		Y	•	•	A	L		P	L
FYN	TK	•	•			•	•		Y		•			•	•	•
IGF1R	TK	•		•	EV	•	FD	•	Y	•	S		T	•	K	
JAK2	TK	S	L	•	•		D	•	Y	L	K			•	•	•
LCK	TK	•	•	•		D	D		Y					•	•	•
LYN	TK	E	E	E	•	•	•		Y	E	•	L	D	•	P	
MET	TK	S	•	D		D	E	•	Y	V	P	V	•	P	•	•
PDGFRB	TK	•	•	•	•	D	•	•	Y	V	•	I		P	•	•
PTK2	TK	•	•	•	E				Y	•	•		E	E	D	D
RET	TK	T	R		SE	•	•		Y		•	•	GS	•		R
SRC	TK	•	•	•	•	•	•		Y	•	•			•	•	•
SYK	TK	E	E	E	D	DE	D	D	Y	E	•	P	•	E	•	•
YES1	TK	•	•	IPE	E		•	•	Y	E	A	P	•	•		K
ZAP70	TK		P	E	D	DE	•	D	Y	E	•	VP	P	E	•	E

Acidic Basic Neutral
Ser/Thr Kink Hydrophobic

Figure 2

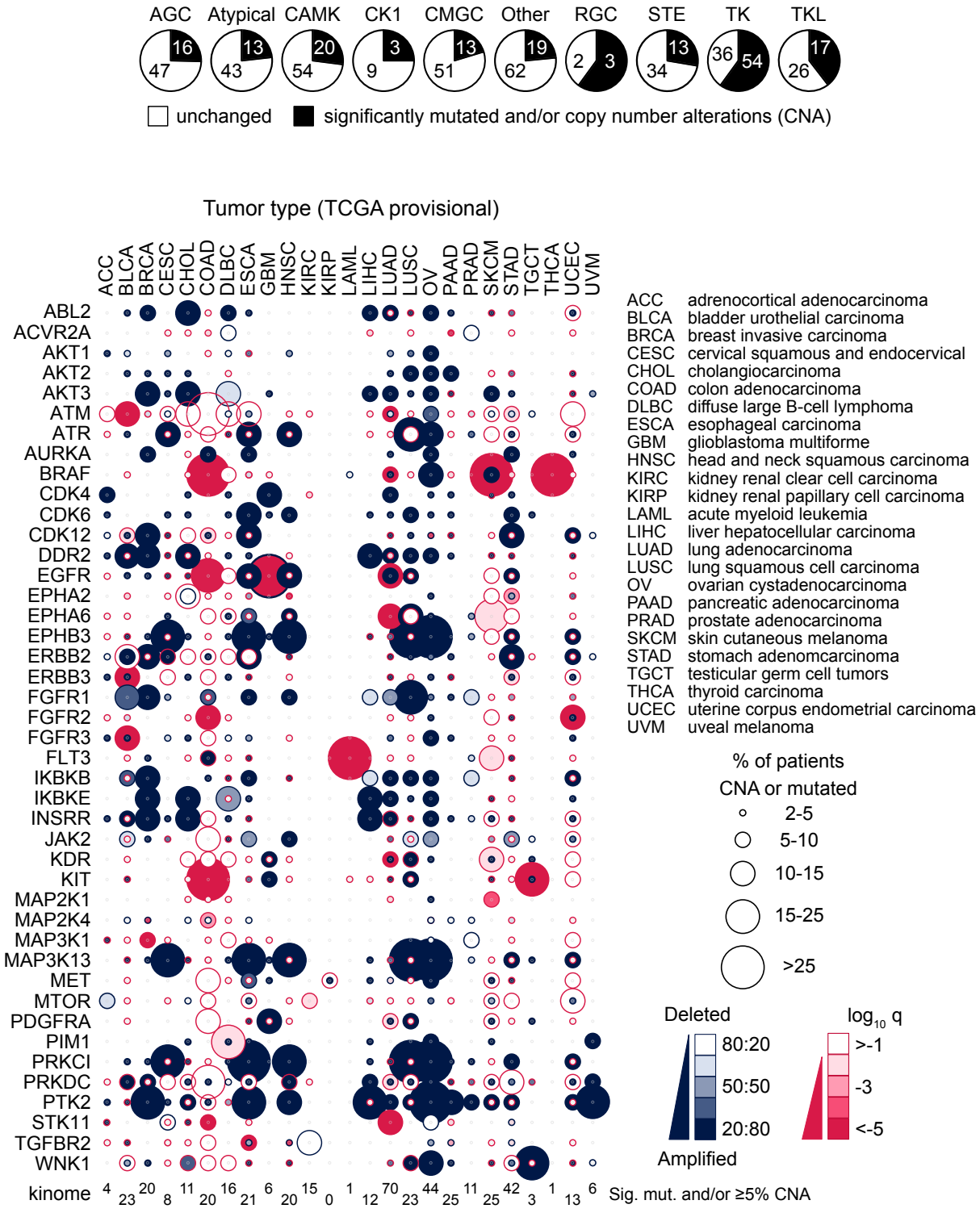
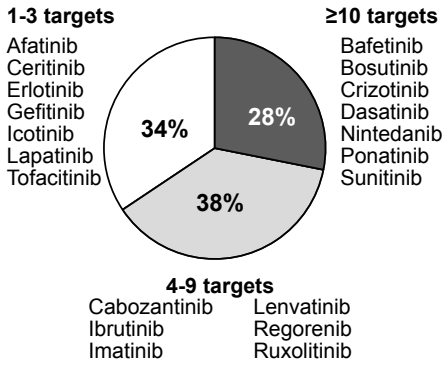

AGC
Atypical
CAMK
CK1
CMGC
Other
RGC
STE
TK
TKL
16
47
13
43
20
54
3
9
13
51
19
62
2
3
13
34
36
54
17
26
unchanged
significantly mutated and/or copy number alterations (CNA)
Tumor type (TCGA provisional)
ACC BLCA BRCA CESC CHOL COAD DLBC ESCA GBM HNSC KIRC KIRP LAML LIHC LUAD LUSC OV PAAD PRAD SKCM STAD TGCT THCA UCEC UVM
ABL2
ACVR2A
AKT1
AKT2
AKT3
ATM
ATR
AURKA
BRAF
CDK4
CDK6
CDK12
DDR2
EGFR
EPHA2
EPHA6
EPHB3
ERBB2
ERBB3
FGFR1
FGFR2
FGFR3
FLT3
IKBKB
IKBKE
INSRR
JAK2
KDR
KIT
MAP2K1
MAP2K4
MAP3K1
MAP3K13
MET
MTOR
PDGFRA
PIM1
PRKCI
PRKDC
PTK2
STK11
TGFBR2
WNK1
kinome
4 23 20 8 11 20 16 21 6 20 15 0 1 12 70 23 44 25 11 25 42 3 1 13 6
Sig. mut. and/or ≥5% CNA
ACC adrenocortical adenocarcinoma
BLCA bladder urothelial carcinoma
BRCA breast invasive carcinoma
CESC cervical squamous and endocervical
CHOL cholangiocarcinoma
COAD colon adenocarcinoma
DLBC diffuse large B-cell lymphoma
ESCA esophageal carcinoma
GBM glioblastoma multiforme
HNSC head and neck squamous carcinoma
KIRC kidney renal clear cell carcinoma
KIRP kidney renal papillary cell carcinoma
LAML acute myeloid leukemia
LIHC liver hepatocellular carcinoma
LUAD lung adenocarcinoma
LUSC lung squamous cell carcinoma
OV ovarian cystadenocarcinoma
PAAD pancreatic adenocarcinoma
PRAD prostate adenocarcinoma
SKCM skin cutaneous melanoma
STAD stomach adenomcarcinoma
TGCT testicular germ cell tumors
THCA thyroid carcinoma
UCEC uterine corpus endometrial carcinoma
UVM uveal melanoma
% of patients
CNA or mutated
2-5
5-10
10-15
15-25
>25
Deleted
80:20
50:50
20:80
Amplified
log10 q
>-1
-3
<-5

Figure 3

Chemical inhibitors	Approved	Target(s)
Sirolimus	1999	MTOR
Gefitinib	2002	EGFR
Imatinib	2002	BCR-ABL, KIT, DDR1
Erlotinib	2005	EGFR, GAK
Sorafenib	2005	many inc. RAF kinases
Dasatinib	2006	SRC family and others
Sunitinib	2006	tyrosine kinases and others
Lapatinib	2007	EGFR, ERBB2
Nilotinib	2007	BCR-ABL, DDR1
Temsirolimus	2007	MTOR
Everolimus	2009	MTOR
Pazopanib	2009	PDGFR, KIT, VEGFR
Cabozantinib	2011	VEGFR, MET, RET, KIT
Crizotinib	2011	ALK, ROS1, MET, LCK, etc
Icotinib	2011	EGFR
Ruxolitinib	2011	JAKs, TYK2
Vandetanib	2011	EGFR, VEGFR
Vemurafenib	2011	BRAF mutant, RAF1
Axitinib	2012	VEGFR, PDGFR, KIT, etc
Lenvatinib	2012	VEGFR, PDGFR
Ponatinib	2012	RTKs, SRC family kinases etc
Regorafenib	2012	VEGFR, TIE2, KIT, RET, etc
Tofacitinib	2012	JAK2, JAK3
Afatinib	2013	EGFR, ERBB2, ERBB4
Bosutinib	2013	tyrosine kinases and others
Dabrafenib	2013	BRAF, RAF1
Ibrutinib	2013	BTK, EGFR
Trametinib	2013	MAP2K1, MAP2K2
Bafetinib	2014	BCR-ABL, LYN, FYN
Ceritinib	2014	ALK, INSR, IGF1R
Nintedanib	2014	tyrosine kinases and others
Alectinib	2015	ALK
Cobimetinib	2015	MAP2K1
Osimertinib	2015	mutant EGFR
Palbociclib	2015	CDK4, CDK6
Midostaurin	2017	FLT3, KIT

mAb inhibitors	Approved	Target(s)
Trastuzumab	1998	anti-ERBB2
Panitumumab	2006	anti-EGFR
Cetuximab	2009	anti-EGFR
Pertuzumab	2012	anti-ERBB2
Ramucirumab	2014	anti-KDR
Necitumumab	2015	anti-EGFR
Olaratumab	2016	anti-PDGFR
Agonists	**Approved**	**Target(s)**
Insulin	1920s, 1982	INSR
Becaplermin	1997	PDGFR
Palifermin	2004	FGFR2
Mecasermin	2005	IGF1R
Linaclotide	2012	GUCY2C
Ligand modulators	**Approved**	**Target(s)**
Bevacizumab	2004	anti-VEGF
Pegaptanib	2004	anti-VEGF
Ranibizumab	2006	anti-VEGF
Aflibercept	2011	anti-VEGF

FDA approved chemical inhibitors



(≤50nM IC_{50}/K_D)

Figure 4

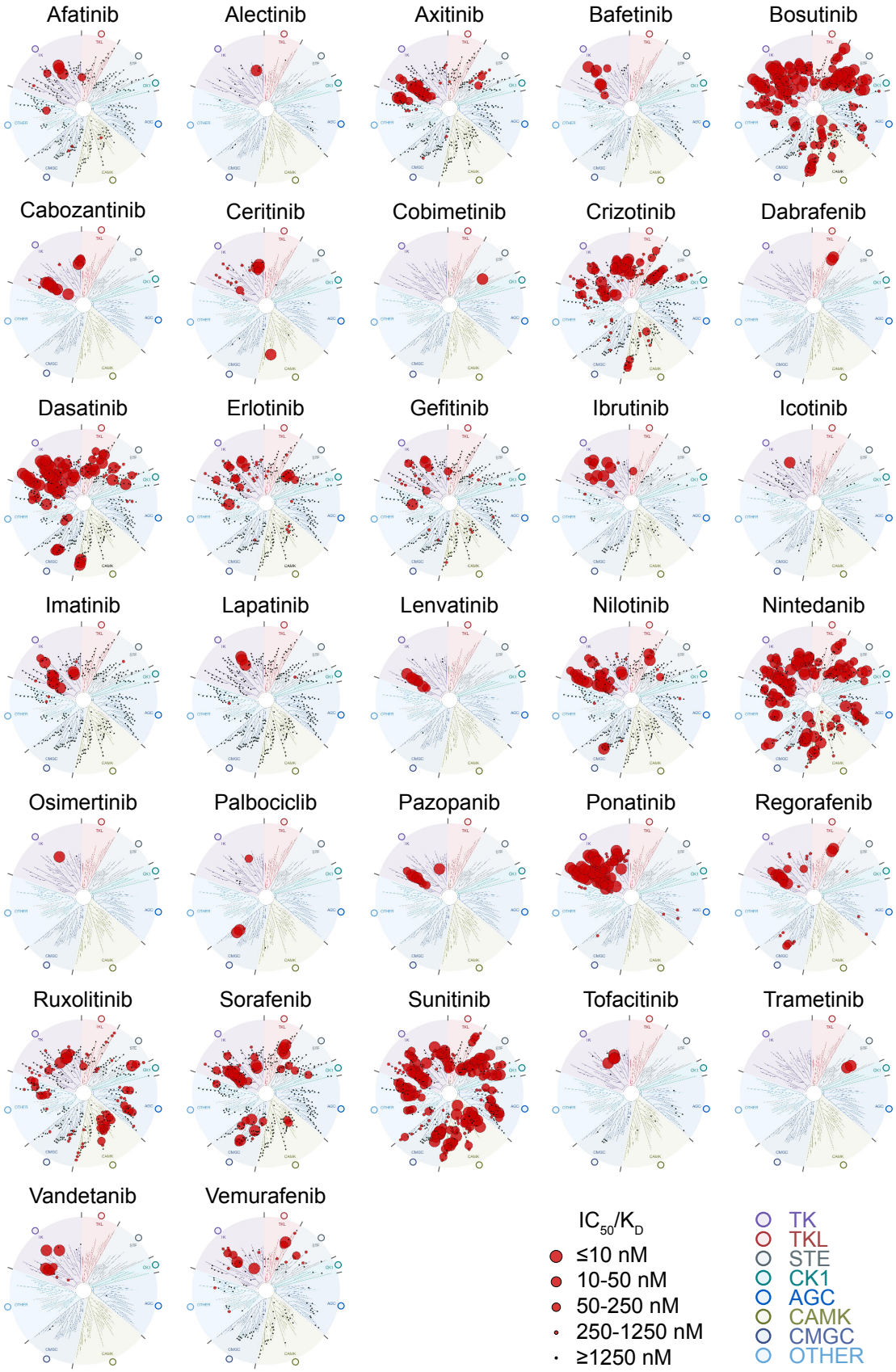

Afatinib
Alectinib
Axitinib
Bafetinib
Bosutinib
Cabozantinib
Ceritinib
Cobimetinib
Crizotinib
Dabrafenib
Dasatinib
Erlotinib
Gefitinib
Ibrutinib
Icotinib
Imatinib
Lapatinib
Lenvatinib
Nilotinib
Nintedanib
Osimertinib
Palbociclib
Pazopanib
Ponatinib
Regorafenib
Ruxolitinib
Sorafenib
Sunitinib
Tofacitinib
Trametinib
Vandetanib
Vemurafenib
IC50/KD
≤10 nM
10-50 nM
50-250 nM
250-1250 nM
≥1250 nM
TK
TKL
STE
CK1
AGC
CAMK
CMGC
OTHER

Figure 5

Kinome ontology/overview	
KinBASE	http://www.kinase.com/
KinaseNET	http://www.kinasenet.ca/
ProKinO	http://vulcan.cs.uga.edu/prokino
Phosphorylation/kinase-substrate resources	
GPS 3.0	http://gps.biocuckoo.org/
HPRD	http://www.hprd.org/
Phospho.ELM	http://phospho.elm.eu.org/
PHOSIDA	http://141.61.102.18/phosida/index.aspx
PhoshoNET	http://www.phosphonet.ca/
PhosphoNetworks	http://www.phosphonetworks.org/
PhosphoSitePlus	https://www.phosphosite.org
RegPhos 2.0	http://140.138.144.141/~RegPhos/index.php
Kinase drug sensitivity and affinity measurements	
canSAR 3.0	http://cansar.icr.ac.uk
ChEMBL	https://www.ebi.ac.uk/chembldb/
Drugbank	https://www.drugbank.ca/
DrugKiNET	http://www.drugkinet.ca/
Kinase SARfari	https://www.ebi.ac.uk/chembl/sarfari/kinasesarfari
International Centre for Kinase Profiling	http://www.kinase-screen.mrc.ac.uk/
Disease associations	
COSMIC	http://cancer.sanger.ac.uk/cosmic
DECIPHER	http://diseases.jensenlab.org
DISEASES	https://decipher.sanger.ac.uk/
TCGA	https://cancergenome.nih.gov/
UniProtKB	http://www.uniprot.org/uniprot/

Table 1. *Kinase databases and resources*
Links to currently available web resources relevant to kinome and kinase biology.