**Chapter 1: Targeting kinases for cancer treatment**

* 1. **History of kinase inhibition for cancer treatment**

Protein kinases are enzymes that utilize adenosine triphosphate (ATP) as a co-factor to transfer phosphoryl groups onto other protein substrates, primarily on serine, threonine or tyrosine residues. Like other post-translational modifications, the addition of a phosphoryl group can induce changes in protein structure, cellular localization, protein-protein interactions and/or enzymatic activities.

The first descriptions of protein phosphorylation occurred in the mid-1950s1, 2, in which the activity of glycogen phosphorylase was found to be modulated by glycogen phosphorylase kinase. Although that was initially considered to be an isolated example, protein phosphorylation is now widely accepted to be an integral component of all cellular processes3. The rapid and reversible nature of protein phosphorylation, in addition to its ability to control protein functions, makes it an ideal mechanism by which cells can control the states and amplitudes of signaling pathways in response to internal and external stimuli.

Many diseases, including cancer, are caused by dysregulated protein activities and/or abnormal activation of signaling pathways. Although we now know that kinases regulate critical signaling pathways, and thus are good candidates as targets to ameliorate diseases, it was not until 1978 when Ray Erikson identified the transforming factor of the Rous sarcoma virus (v-Src) as a protein kinase4 that people began to appreciate the possibility that deregulated kinase activities could be the root cause of many cancers. Subsequent discoveries uncovered many other crucial roles for kinases in tumorigenesis. For example, growth factor receptors, such as the epidermal growth factor receptor (EGFR)5, contain intracellular tyrosine kinase domains that transmit growth and differentiation signals across the membrane to downstream effectors, and constitutive activation of EGFR may lead to uncontrolled cellular growth. In addition, cell cycle progression, another key aspect of proliferation that is dysregulated in transformed cells, was found to be controlled by various kinases, including the cyclin-dependent kinases (CDKs)6. As our understanding of the role of kinases in promoting cellular transformation and tumorigenesis increased, small molecules capable of inhibiting the activity of these oncogenic kinases were widely pursued as potential treatments for cancer, and now have become the largest class of drugs for oncology indications7.

* 1. **Current use of kinase inhibitors in oncology**

In May 2001, imatinib (Gleevec) was the first kinase inhibitor to gain Food and Drug Administration (FDA) approval, specifically for the treatment of patients with Philadelphia chromosome-positive chronic myelogenous leukemia (CML) that expresses the oncogenic breakpoint cluster region-Abelson tyrosine kinase (BCR-ABL) fusion gene8. This milestone was followed by a steady stream of approval of kinase inhibitors, roughly one per year for the first decade of the twenty-first century9. Since 2011, the approval rate has increased significantly, with 24 of the 32 approved kinase inhibitors for cancer treatment receiving their nods from the FDA during this period (Table 1.1). This boom was likely driven by a number of factors, including: (1) advancement in technologies, such as whole genome sequencing, which allowed for the stratification of patient populations to identify responsive subgroups10; (2) regulatory policies that expedited the drug development process and accelerated approval11, 12; (3) accumulated experience from both academia and industry in the design of kinase inhibitors with the help of X-ray crystallography and high-throughput screening platforms; and probably most importantly, (4) a deeper understanding of the underlying biological mechanisms that drive tumor growth.

However, the primary kinase targets of 76% of the FDA-approved kinase inhibitors—with the caveat that many of the inhibitors are multi-targeted and exhibit polypharmacology13—are tyrosine kinases, which are further overrepresented by BCR-ABL (5 inhibitors), EGFR/HER2 (6 inhibitors), vascular endothelial growth factor receptors (VEGFRs; 8 inhibitors) and anaplastic

**Table 1.1. Overview of 33 FDA-approved kinase inhibitors for cancer treatment**

|  |  |  |  |
| --- | --- | --- | --- |
| **Drug** | **Primary Kinase Targets** | **Approved Indications** | **Year of Approval** |
| Imatinib | BCR-ABL | KIT | PDGFR | Ph+ CML and ALL, GIST | 2001 |
| Gefitinib | EGFR |  |  | EGFR-mutant NSCLC | 2003 |
| Erlotinib | EGFR | PDGFR | KIT | EGFR-mutant NSCLC | 2004 |
| Sorafenib | VEGFR | PDGFR | BRAF | RCC, Thyroid Cancer | 2005 |
| Sunitinib | VEGFR | PDGFR | KIT | RCC, GIST | 2006 |
| Dasatinib | BCR-ABL | SRC-family | KIT | Ph+ CML, Ph+ ALL | 2006 |
| Lapatinib | EGFR | HER2 |  | HER2+ Breast Cancer | 2007 |
| Nilotinib | BCR-ABL | PDGFR | KIT | Ph+ CML | 2007 |
| Pazopanib | VEGFRs | PDGFR | FGFR | RCC, Soft Tissue Sarcoma | 2009 |
| Vendatanib | VEGFR | EGFR | RET | Medullary Thyroid Carcinoma | 2011 |
| Vemurafenib | RAF | SRMS | ACK1 | BRAF-mutant Melanoma | 2011 |
| Crizotinib | ALK | MET |  | ALK-positive NSCLC | 2011 |
| Ruxolitinib | JAK1 | JAK2 |  | Polycythemia Vera | 2011 |
| Axitinib | VEGFRs | KIT | PDGFR | RCC | 2012 |
| Bosutinib | BCR-ABL | SRC-family |  | Ph+ CML | 2012 |
| Regorafenib | VEGFR2 | TIE2 |  | CRC, GIST | 2012 |
| Cabozantinib | VEGFRs | MET | RET | Thyroid Cancer, RCC | 2012 |
| Ponatinib | BCR-ABL | VEGFR | SRC | Ph+ CML, Ph+ ALL | 2012 |
| Trametinib | MEK1 | MEK2 |  | BRAF-mutant Melanoma | 2013 |
| Dabrafenib | BRAF | CRAF | MEK | BRAF-mutant Melanoma | 2013 |
| Afatinib | EGFR | HER2 | HER4 | EGFT-mutant NSCLC | 2013 |
| Ibrutinib | BTK |  |  | CLL, Mantle Cell Lymphoma, WM | 2013 |
| Ceritinib | ALK | IGF1R | INSR | ALK+ NSCLC | 2014 |
| Idelalisib | PK3CD |  |  | CLL, Follicular B-cell NHL. | 2014 |
| Palbociclib | CDK4 | CDK6 |  | HER2- ER+ Breast Cancer | 2015 |
| Lenvatinib | VEGFRs | PDGFR | KIT | Thyroid Cancer, RCC | 2015 |
| Cobimetinib | MEK1 | MEK2 |  | BRAF-mutant Melanoma | 2015 |
| Osimertinib | EGFR |  |  | EGFR-mutant(T790M) NSCLC  | 2015 |
| Alectinib | ALK | RET |  | ALK+ NSCLC | 2015 |
| Ribociclib | CDK4 | CDK6 |  | HER2- ER+ Breast Cancer | 2017 |
| Brigatinib | ALK | ROS1 | IGF1R | ALK+ NSCLC | 2017 |
| Midostaurin | FLT3 | KIT | PDGFR | FLT3+ AML | 2017 |
| Neratinib | EGFR | HER2 | HER4 | HER2+ Breast Cancer | 2017 |

ALL, acute lymphoblastic leukemia; AML, acute myeloid leukemia; CLL, chronic lymphocytic leukemia; CML, chronic myeloid leukemia; CRC, colorectal cancer; GIST, gastrointestinal stromal tumors; NHL, non-Hodgkin lymphoma; NSCLC, non-small-cell lung cancer; Ph, Philadelphia chromosome; RCC, renal cell carcinoma; WM, Waldenström’s macroglobulinemia.

lymphoma kinase (ALK; 4 inhibitors). As there are 518 protein kinases encoded in the human genome14, it is clear that we have not exploited the full potential of kinases as a target class.

However, the primary kinase targets of 76% of the FDA-approved kinase inhibitors—with the caveat that many of the inhibitors are multi-targeted and exhibit polypharmacology13—are tyrosine kinases, which are further overrepresented by BCR-ABL (5 inhibitors), EGFR/HER2 (6 inhibitors), vascular endothelial growth factor receptors (VEGFRs; 8 inhibitors) and anaplastic lymphoma kinase (ALK; 4 inhibitors). As there are 518 protein kinases encoded in the human genome14, it is clear that we have not exploited the full potential of kinases as a target class.

Kinases that have been successful targets in oncology can be divided into three categories, based on their biological roles in promoting tumorigenesis15. Kinases in the first group actively drive cancer progression, and usually acquire their dysregulated function through activating mutations, gene amplifications, overexpression or gene fusions16. The absolute requirement of the unregulated activities of these kinases by some cancer cells for survival, or so-called “oncogene addiction,” explains the therapeutic window for inhibitors that target these kinases. Furthermore, genomic characterization of large cohorts of patient tumor samples to look for common mutations, copy number variants and chromosomal rearrangements have allowed for the discovery of these driver kinases16. As many of these kinases were among the first to be identified to play important roles in cancer5, 17, 18, the majority of clinical kinase targets fall in this category. Unfortunately, development of resistance to these inhibitors is extremely common, as a single point mutation (e.g. EGFR(T790M)19, BCR-ABL(T315I)20) can render the inhibitors ineffective and thereby restore kinase function. Alternatively, cancer cells can adapt to the treatment via activation of downstream signaling components or parallel pathways to sustain their own proliferation and survival.

The second class of kinase target represents dependencies that arise due to abnormal cellular processes and altered genetic contexts acquired by tumor cells for growth and survival. These targets are generally not oncogenic or mutated themselves, but are associated with a dysregulated pathway, or are functionally associated with certain hallmarks of cancer21, 22. For example, targeting mitogen-activated protein kinase kinase 1/2 (MAP2K1/2, or MEK1/2) in metastatic v-raf murine sarcoma viral oncogene homolog B (BRAF)-mutant melanoma with upregulated mitogen-activated protein kinase (MAPK) pathway activities has been proven effective as both a single agent and in combination with BRAF inhibitors23, 24. Targeting the DNA-damage response machineries25 (e.g. ataxia telangiectasia mutated (ATM) and ataxia telangiectasia and Rad3-related protein (ATR)) in cancers with genomic instability, or targeting cell cycle kinases in cancers with abnormal ploidy represent other possibilities26.

The third class of kinase targets is comprised of both intrinsic and extrinsic factors of tumor cells that are necessary for the progression of the disease, including adequate blood supply, metastasis, tumor-promoting inflammation, and evasion of the immune system. Examples include VEGFR inhibitors that disrupt tumor angiogenesis. These targets are more difficult to study, as context-specific assays instead of simple proliferation assays are needed to evaluate the potential of targeting these kinases, making drug development efforts significantly more challenging. Furthermore, adequate *in vitro* and *in vivo* tumor models may not exist to accurately assess the potential of inhibiting these targets in humans, further impeding the validation of the clinical efficacy of these kinases.

Overcoming clinical resistance and accelerating the discovery of novel targets to address unmet clinical needs are major challenges we currently face in clinical oncology. Branching out from the growth-promoting tyrosine kinase targets, which have been widely studied, to the second and third classes of kinase targets will offer new opportunities for combination therapies to prevent or delay the emergence of resistance. Furthermore, targeting context-specific kinase dependencies may provide better therapeutic windows than the ones that target proliferation pathways, increasing the tolerability of cancer medicine and improving patient care.

* 1. **AMPK-related kinase family**

Among the understudied territories within the kinome, the AMPK (AMP-activated protein kinase)-related kinase family, including brain-specific kinase 1 (BRSK1), BRSK2, novel (nua) kinase 1 (NUAK1), NUAK2, microtubule-associated protein (MAP)/microtubule affinity-regulating kinase 1 (MARK1), MARK2, MARK3, MAKR4, salt-inducible kinase 1 (SIK1), SIK2, SIK3 and maternal embryonic leucine zipper kinase (MELK), have been implicated in various roles, including brain development, energy homeostasis, tumorigenesis and innate immunity27. By sequence homology, the kinase domains of the AMPK-related kinases are similar to the catalytic domain of AMPK14, which exists as a trimeric protein kinase complex that senses energy balance and regulates metabolism at both the cellular and the physiologic levels28. Furthermore, liver kinase B1 (LKB1, or STK11) in complex with STE20-related kinase adaptor (STRAD) and calcium-binding protein 39 (CAB39, or MO25) activates AMPK and AMPK-related kinases (except for MELK) by phosphorylating a threonine residue at an equivalent position in their activation loops29. Another structural similarity of the AMPK-related kinases is that all members, except NUAK1 and NUAK2, contain a ubiquitin-association domain (UBA) immediately C-terminal to the kinase domain. These UBA domains in the AMPK-related kinases do not exhibit affinity towards ubiquitin, polyubiquitin chains, or ubiquitin-like proteins, but play essential structural roles in maintaining the catalytic function of the kinases30.

 MARKs primarily phosphorylate microtubule-associated proteins such as tau, MAP2, MAP431 and doublecortin32 on the KXGS motif, causing them to detach from microtubules. These changes in turn affect the stability of microtubules31, vesicle transport33, neurite outgrowth34 and neuron mobility32. In addition, MARK1 and 2 are involved with the establishment of cell polarity, and are asymmetrically localized in epithelial cells35. These properties are consistent with the function of the MARK ortholog PAR-1 (partitioning-defective 1) in *C. elegans*, which was identified as an essential gene for establishing early embryonic polarity36. Apart from these functions involving cytoskeletal dynamics and cell polarity, MARK3 has been shown to phosphorylate and inhibit cell division cycle 25C (CDC25C) phosphatase, preventing mitotic entry37. Almost exclusively expressed in the brain, BRSK1 and 2 share many similar neuronal functions as MARKs, including phosphorylation of tau, neuronal polarization and exon specification38, 39.

SIK1-3 are involved in the regulation of innate immunity and cytokine production40-43, glucose and lipid metabolism44-47 and melanin production48. As diverse as these functions may be, the primary proposed mechanism of these regulations involves SIK-dependent phosphorylation of the cAMP-responsive element-binding protein (CREB)-regulated transcription coactivators40-48 (CRTCs) and/or the class IIa histone deacetylases41, 43, 44, 49 (HDACs), which causes sequestration of these transcription regulators by 14-3-3 proteins in the cytosol and cessation of their respective transcription programs.

 NUAK1 and 2 have also been reported to regulate cytoskeletal dynamics, which may in turn affect diverse phenotypes including cell adhesion50, neural tube formation51 and axon branching52. Furthermore, NUAK1 has been widely suggested to promote tumorigenesis, conferring resistance to nutrient deprivation53, 54 and promoting chemoresistance55, cell invasion and metastasis56-58. NUAK2 likely presents some redundant functions as NUAK1, as only NUAK1/2 double knockout confer neural tube defects in mice with full penetrance51. Also, both kinases phosphorylate myosin phosphatase targeting-1 (MYPT1) to control myosin light chain dynamics50. However, their importance may be different in a context-specific manner, especially in the case of cancer. Unfortunately, the molecular mechanisms that mediate most of their reported physiological and pathological functions remain poorly understood.

MELK was first identified as a maternal transcript in mouse embryos, and was thought to play an important role during early embryonic development59, 60. Interestingly, germ-line knockout of *MELK* in mice caused no apparent effect on health and fertility61, 62. MELK expression is negligible in most adult tissues63, 64, but is often found in progenitor cells65, 66, suggesting a proliferation- and self-renewal-related function. Most importantly, MELK is overexpressed in multiple cancers, and multiple reports have utilized RNA interference (RNAi) methods to suggest that depletion of MELK protein levels impairs the growth of MELK-expressing cancer cell lines62, 64, 67-70. Putative substrates of MELK include cell division cycle 25B (CDC25B)71, apoptosis signal-regulating kinase 1 (ASK1)72, zinc-finger-like protein 9 (ZRP9)73, 74, apoptosis facilitator Bcl-2-like protein 14 (BCL2L14, or BCL-G)64 and eukaryotic translation initiation factor 4B (EIF4B)75, suggesting that MELK might be involved with cell cycle progression, resistance to apoptosis, transcription factor regulation and protein translation. However, most of these interactions remain poorly elucidated, have not been supported by further reports, and do not offer a consistent mechanism to explain the potential oncogenic role of MELK.

Nevertheless, there is suggestive evidence that members of the AMPK-related kinase family play an important role in tumorigenesis. For example, as loss of function in the LKB1 gene causes Peutz-Jeghers Syndrome, a hereditary disorder that predisposes patients to the development of benign and malignant tumors76, 77, many have wondered if dysregulation of AMPK and other AMPK-related kinases contribute to this phenotype. Furthermore, NUAK1/2 and MELK have been associated with various roles in promoting survival, proliferation and metastasis of cancer cells, even though the underlying mechanisms of their roles are poorly understood. Thus, the AMPK-related kinase family is a clear example of a group of understudied kinases whose biological roles require further elucidation. Development of selective kinase inhibitors targeting each AMPK-related kinases will be especially beneficial to determine their respective biological functions and therapeutic potential. In **Chapter 2**, I describe my efforts to design selective MELK inhibitors and to validate MELK as a therapeutic target for basal-like breast cancers.

* 1. **Rationally designed kinase degraders**

In addition to targeting new kinases for the treatment of cancer, developing new modes of actions for small molecule drugs is an important approach to add to our existing arsenal of cancer therapeutics. Recently, heterobifunctional molecules that recruit E3 ligases into close proximity with proteins of interest, thereby inducing their ubiquitination and degradation, have emerged as a promising new class of drugs78, 79. Unlike compounds that require active site or allosteric site occupancy to inhibit enzymatic activities, protein degraders catalytically induce target ubiquitination, so a high degree of target occupancy is not necessary to maintain efficacy. Furthermore, protein degraders counteract target upregulation as a potential resistance mechanism and may exhibit a more durable response, since protein re-synthesis takes time.

The idea of using bivalent molecules to hijack the ubiquitin proteosome system for protein degradation was pioneered by Crews and Deshaies more than a decade ago, when they introduced the proteolysis-targeting chimeras (PROTACs)80. The first iteration of PROTACs (PROTAC-1) utilized a phosphopeptide from NF-κB inhibitor alpha (IκBα) that bound specifically to an F-box protein β-transducin repeat-containing protein (β-TRCP) in the S-phase kinase-associated protein 1 (SKP1)-cullin 1-F-box-protein E3 ligase complex (SCFβ-TRCP) for E3 ligase recruitment. While *in vitro* reconstitution of the system demonstrated the feasibility of recruiting SCFβ-TRCP to mediate target ubiquitination, the phosphopeptidic nature of the first generation of PROTACs led to issues including poor cellular uptake and poor stability, which limited its use in cell culture and *in vivo*.

In an effort to design PROTACs with more drug-like properties, chemical ligands for the E3 ligase mouse double minute 2 homolog (MDM2) and cellular inhibitor of apoptosis 1 (cIAP1; cIAP1-based degraders were termed specific and non-genetic inhibitor of apoptosis protein-dependent protein erasers, or SNIPERs) were utilized to degrade androgen receptor (AR)81, 82, estrogen receptor (ER)81 and cytosolic retinoic acid-binding protein I (CRABPI) and CRABPII83. However, the cellular potency of these agents was still in the micromolar range. Furthermore, the cIAP1 ligand bestatin presented off-target activities on arginyl aminopeptidases and leukotriene A4 hydrolase84, 85, and induced cIAP1 autoubiqutination and degradation86, both of which limited the applicability of this strategy. Recently, SNIPERs targeting AR or BCR-ABL based on a specific IAP ligand derived from LCL-161 were reported, and the ABL-targeting compound SNIPER(ABL)-39 demonstrated low-nanomolar potency in cellular assays87, 88. However, cIAP1 degradation induced by the cIAP1 ligand is still a limiting issue.

The hydrophobic tagging (HyT) strategy, which utilizes either adamantane89-91 or Boc3Arg92 moieties to elicit an unfolded protein response and subsequent target degradation, was demonstrated to be an effective strategy against HaloTag fusion proteins90 and clinically-relevant targets including a psuedokinase human epidermal growth factor receptor 3 (HER3)91. However, the HyT strategy will likely remain a chemical tool due to the relatively ambiguous molecular mechanism of the HyT strategy and concerns related to the chemical and biological properties of adamantane and Boc3Arg.

The primary breakthroughs that transformed small molecule-induced protein degradation into a potentially viable therapeutic approach took place in 2015, when several reports described efficient degraders that operate in the mid-to-low nanomolar range, and with *in vivo* efficacy93-96. These breakthroughs were enabled by the discovery that the E3 ligase cereblon (CRBN) is the molecular target of the teratogen and immunomodulatory imide drug (IMiD) thalidomide in 201097, and the development of a potent and specific chemical ligand for the E3 ligase von Hippel-Lindau (VHL) in 201298-100. The increased drug-like properties of these E3 ligase ligands compared with the previous versions greatly improved the potency, stability and permeability of the degrader molecules. Moreover, induced degradation was found in some cases to have more potent effects than inhibition. For example, a CRBN-mediated bromodomain-containing protein 4 (BRD4) degrader, termed dBET1, outperformed its parental BRD4 inhibitor JQ1 in inhibiting proliferation of acute myeloid leukemia (AML) in a disseminated AML model in mice95. A VHL-mediated estrogen-related receptor alpha (ERRα) degrader, termed PROTAC\_ERRα, also demonstrated efficient ERRα degradation in mouse heart and kidney, and in xenographic MDA-MB-231 tumors93. These promising pre-clinical results spurred the founding of Arvinas (New Haven, Connecticut; 2013) and C4 Therapeutics (Cambridge, Massachusetts; 2015), both of which focus on hijacking the ubiquitin proteosome system for therapeutic benefits.

Despite the clear potential of bivalent degrader drugs, this class of compounds faces several challenges. First of all, despite improvements on the cell penetration properties, poor cellular uptake remains an issue for the degraders, which will be even more challenging for clinical translation. Second, the structure activity relationship for linker length and linker chemistry is not well understood, partly due to the infancy of the field. While it is not difficult to imagine that an optimal length of linkers should exist that will mediate the target-E3 ligase interactions optimally, linkers may fold and be involved in the induced protein-protein interaction101. Adding in the possibilities of changing the atomic composition and the rigidity of the linkers to improve the pharmacokinetic and pharmacodynamic properties, linker design becomes a heavily empirical step. Third, reports have described widely divergent degradation efficiencies against BCR-ABL when different ABL-targeting ligands and E3 ligase-recruiting ligands were combined87, 102. Those results informed the need to survey variations in both ends of a degrader molecule; however, there is limited molecular understanding of the degrader system to explain and predict outcomes.

The third point contributes to this last question, which is whether the degrader strategy is simply modular and applicable to all “ligandable” targets. In other words, are all targets amenable to degradation as long as we can design a degrader that can bind the respective ends? The answer is likely no, as several reports have demonstrated that converting an inhibitor into a degrader improves selectivity93, 96, 102. For example, the VHL-mediated BRD degrader MZ1 preferentially degraded BRD4 over BRD2 and BRD3 despite comparable affinities for the bromodomains of all three proteins, and the selectivity was attributed the differential cooperativity for complex formation between the VHL complex and the different bromodomains96. In addition, BCR-ABL is readily degraded by CRBN-mediated but not VHL-mediated degraders, signifying that different E3 ligases exhibit different abilities to degrade *de novo* substrates102. To systematically investigate if some targets are more tractable to the degrader strategy than others, in **Chapter 3**, we used a multiplexed quantitative proteomic method in combination with a multi-kinase degrader to survey the degradable kinome. The goals of this study were to broaden our understanding of small molecule degraders as a drug class and to rapidly identify degradable kinases in order to prioritize medicinal chemistry efforts for those with clear therapeutic implications.

* 1. **References**

1. Fischer, E.H. & Krebs, E.G. Conversion of phosphorylase b to phosphorylase a in muscle extracts. *J Biol Chem* **216**, 121-132 (1955).

2. Sutherland, E.W., Jr. & Wosilait, W.D. Inactivation and activation of liver phosphorylase. *Nature* **175**, 169-170 (1955).

3. Cohen, P. The origins of protein phosphorylation. *Nat Cell Biol* **4**, E127-130 (2002).

4. Collett, M.S. & Erikson, R.L. Protein kinase activity associated with the avian sarcoma virus src gene product. *Proc Natl Acad Sci U S A* **75**, 2021-2024 (1978).

5. Ushiro, H. & Cohen, S. Identification of phosphotyrosine as a product of epidermal growth factor-activated protein kinase in A-431 cell membranes. *J Biol Chem* **255**, 8363-8365 (1980).

6. Lapenna, S. & Giordano, A. Cell cycle kinases as therapeutic targets for cancer. *Nat Rev Drug Discov* **8**, 547-566 (2009).

7. Kinch, M.S. An analysis of FDA-approved drugs for oncology. *Drug Discov Today* **19**, 1831-1835 (2014).

8. Druker, B.J. *et al.* Efficacy and safety of a specific inhibitor of the BCR-ABL tyrosine kinase in chronic myeloid leukemia. *N Engl J Med* **344**, 1031-1037 (2001).

9. Wu, P., Nielsen, T.E. & Clausen, M.H. FDA-approved small-molecule kinase inhibitors. *Trends Pharmacol Sci* **36**, 422-439 (2015).

10. Biankin, A.V., Piantadosi, S. & Hollingsworth, S.J. Patient-centric trials for therapeutic development in precision oncology. *Nature* **526**, 361-370 (2015).

11. Kesselheim, A.S., Wang, B., Franklin, J.M. & Darrow, J.J. Trends in utilization of FDA expedited drug development and approval programs, 1987-2014: cohort study. *BMJ* **351**, h4633 (2015).

12. Shea, M. *et al.* Regulatory Watch: Impact of breakthrough therapy designation on cancer drug development. *Nat Rev Drug Discov* **15**, 152 (2016).

13. Li, Y.H. *et al.* The Human Kinome Targeted by FDA Approved Multi-Target Drugs and Combination Products: A Comparative Study from the Drug-Target Interaction Network Perspective. *PLoS One* **11**, e0165737 (2016).

14. Manning, G., Whyte, D.B., Martinez, R., Hunter, T. & Sudarsanam, S. The protein kinase complement of the human genome. *Science* **298**, 1912-1934 (2002).

15. Zhang, J., Yang, P.L. & Gray, N.S. Targeting cancer with small molecule kinase inhibitors. *Nat Rev Cancer* **9**, 28-39 (2009).

16. Gross, S., Rahal, R., Stransky, N., Lengauer, C. & Hoeflich, K.P. Targeting cancer with kinase inhibitors. *J Clin Invest* **125**, 1780-1789 (2015).

17. de Klein, A. *et al.* A cellular oncogene is translocated to the Philadelphia chromosome in chronic myelocytic leukaemia. *Nature* **300**, 765-767 (1982).

18. de Larco, J.E. & Todaro, G.J. Growth factors from murine sarcoma virus-transformed cells. *Proc Natl Acad Sci U S A* **75**, 4001-4005 (1978).

19. Toyooka, S., Kiura, K. & Mitsudomi, T. EGFR mutation and response of lung cancer to gefitinib. *N Engl J Med* **352**, 2136; author reply 2136 (2005).

20. Gorre, M.E. *et al.* Clinical resistance to STI-571 cancer therapy caused by BCR-ABL gene mutation or amplification. *Science* **293**, 876-880 (2001).

21. Hanahan, D. & Weinberg, R.A. The hallmarks of cancer. *Cell* **100**, 57-70 (2000).

22. Hanahan, D. & Weinberg, R.A. Hallmarks of cancer: the next generation. *Cell* **144**, 646-674 (2011).

23. Long, G.V. *et al.* Combined BRAF and MEK inhibition versus BRAF inhibition alone in melanoma. *N Engl J Med* **371**, 1877-1888 (2014).

24. Flaherty, K.T. *et al.* Improved survival with MEK inhibition in BRAF-mutated melanoma. *N Engl J Med* **367**, 107-114 (2012).

25. O'Connor, M.J. Targeting the DNA Damage Response in Cancer. *Mol Cell* **60**, 547-560 (2015).

26. Malumbres, M. & Barbacid, M. Cell cycle kinases in cancer. *Curr Opin Genet Dev* **17**, 60-65 (2007).

27. Bright, N.J., Thornton, C. & Carling, D. The regulation and function of mammalian AMPK-related kinases. *Acta Physiol (Oxf)* **196**, 15-26 (2009).

28. Garcia, D. & Shaw, R.J. AMPK: Mechanisms of Cellular Energy Sensing and Restoration of Metabolic Balance. *Mol Cell* **66**, 789-800 (2017).

29. Lizcano, J.M. *et al.* LKB1 is a master kinase that activates 13 kinases of the AMPK subfamily, including MARK/PAR-1. *EMBO J* **23**, 833-843 (2004).

30. Jaleel, M. *et al.* The ubiquitin-associated domain of AMPK-related kinases regulates conformation and LKB1-mediated phosphorylation and activation. *Biochem J* **394**, 545-555 (2006).

31. Drewes, G., Ebneth, A., Preuss, U., Mandelkow, E.M. & Mandelkow, E. MARK, a novel family of protein kinases that phosphorylate microtubule-associated proteins and trigger microtubule disruption. *Cell* **89**, 297-308 (1997).

32. Schaar, B.T., Kinoshita, K. & McConnell, S.K. Doublecortin microtubule affinity is regulated by a balance of kinase and phosphatase activity at the leading edge of migrating neurons. *Neuron* **41**, 203-213 (2004).

33. Mandelkow, E.M., Thies, E., Trinczek, B., Biernat, J. & Mandelkow, E. MARK/PAR1 kinase is a regulator of microtubule-dependent transport in axons. *J Cell Biol* **167**, 99-110 (2004).

34. Biernat, J. *et al.* Protein kinase MARK/PAR-1 is required for neurite outgrowth and establishment of neuronal polarity. *Mol Biol Cell* **13**, 4013-4028 (2002).

35. Bohm, H., Brinkmann, V., Drab, M., Henske, A. & Kurzchalia, T.V. Mammalian homologues of C. elegans PAR-1 are asymmetrically localized in epithelial cells and may influence their polarity. *Curr Biol* **7**, 603-606 (1997).

36. Kemphues, K.J., Priess, J.R., Morton, D.G. & Cheng, N.S. Identification of genes required for cytoplasmic localization in early C. elegans embryos. *Cell* **52**, 311-320 (1988).

37. Peng, C.Y. *et al.* C-TAK1 protein kinase phosphorylates human Cdc25C on serine 216 and promotes 14-3-3 protein binding. *Cell Growth Differ* **9**, 197-208 (1998).

38. Barnes, A.P. *et al.* LKB1 and SAD kinases define a pathway required for the polarization of cortical neurons. *Cell* **129**, 549-563 (2007).

39. Kishi, M., Pan, Y.A., Crump, J.G. & Sanes, J.R. Mammalian SAD kinases are required for neuronal polarization. *Science* **307**, 929-932 (2005).

40. Clark, K. *et al.* Phosphorylation of CRTC3 by the salt-inducible kinases controls the interconversion of classically activated and regulatory macrophages. *Proc Natl Acad Sci U S A* **109**, 16986-16991 (2012).

41. Sundberg, T.B. *et al.* Development of Chemical Probes for Investigation of Salt-Inducible Kinase Function in Vivo. *ACS Chem Biol* **11**, 2105-2111 (2016).

42. MacKenzie, K.F. *et al.* PGE(2) induces macrophage IL-10 production and a regulatory-like phenotype via a protein kinase A-SIK-CRTC3 pathway. *J Immunol* **190**, 565-577 (2013).

43. Lombardi, M.S., Gillieron, C., Dietrich, D. & Gabay, C. SIK inhibition in human myeloid cells modulates TLR and IL-1R signaling and induces an anti-inflammatory phenotype. *J Leukoc Biol* **99**, 711-721 (2016).

44. Henriksson, E. *et al.* SIK2 regulates CRTCs, HDAC4 and glucose uptake in adipocytes. *J Cell Sci* **128**, 472-486 (2015).

45. Itoh, Y. *et al.* Salt-inducible Kinase 3 Signaling Is Important for the Gluconeogenic Programs in Mouse Hepatocytes. *J Biol Chem* **290**, 17879-17893 (2015).

46. Park, J. *et al.* SIK2 is critical in the regulation of lipid homeostasis and adipogenesis in vivo. *Diabetes* **63**, 3659-3673 (2014).

47. Patel, K. *et al.* The LKB1-salt-inducible kinase pathway functions as a key gluconeogenic suppressor in the liver. *Nat Commun* **5**, 4535 (2014).

48. Mujahid, N. *et al.* A UV-Independent Topical Small-Molecule Approach for Melanin Production in Human Skin. *Cell Rep* **19**, 2177-2184 (2017).

49. Walkinshaw, D.R. *et al.* The tumor suppressor kinase LKB1 activates the downstream kinases SIK2 and SIK3 to stimulate nuclear export of class IIa histone deacetylases. *J Biol Chem* **288**, 9345-9362 (2013).

50. Zagorska, A. *et al.* New roles for the LKB1-NUAK pathway in controlling myosin phosphatase complexes and cell adhesion. *Sci Signal* **3**, ra25 (2010).

51. Ohmura, T., Shioi, G., Hirano, M. & Aizawa, S. Neural tube defects by NUAK1 and NUAK2 double mutation. *Dev Dyn* **241**, 1350-1364 (2012).

52. Courchet, J. *et al.* Terminal axon branching is regulated by the LKB1-NUAK1 kinase pathway via presynaptic mitochondrial capture. *Cell* **153**, 1510-1525 (2013).

53. Suzuki, A. *et al.* ARK5 suppresses the cell death induced by nutrient starvation and death receptors via inhibition of caspase 8 activation, but not by chemotherapeutic agents or UV irradiation. *Oncogene* **22**, 6177-6182 (2003).

54. Liu, L. *et al.* Deregulated MYC expression induces dependence upon AMPK-related kinase 5. *Nature* **483**, 608-612 (2012).

55. Xu, T. *et al.* ARK5 promotes doxorubicin resistance in hepatocellular carcinoma via epithelial-mesenchymal transition. *Cancer Lett* **377**, 140-148 (2016).

56. Obayashi, M. *et al.* microRNA-203 suppresses invasion and epithelial-mesenchymal transition induction via targeting NUAK1 in head and neck cancer. *Oncotarget* **7**, 8223-8239 (2016).

57. Kusakai, G., Suzuki, A., Ogura, T., Kaminishi, M. & Esumi, H. Strong association of ARK5 with tumor invasion and metastasis. *J Exp Clin Cancer Res* **23**, 263-268 (2004).

58. Suzuki, A. *et al.* ARK5 is transcriptionally regulated by the Large-MAF family and mediates IGF-1-induced cell invasion in multiple myeloma: ARK5 as a new molecular determinant of malignant multiple myeloma. *Oncogene* **24**, 6936-6944 (2005).

59. Heyer, B.S., Kochanowski, H. & Solter, D. Expression of Melk, a new protein kinase, during early mouse development. *Dev Dyn* **215**, 344-351 (1999).

60. Heyer, B.S., Warsowe, J., Solter, D., Knowles, B.B. & Ackerman, S.L. New member of the Snf1/AMPK kinase family, Melk, is expressed in the mouse egg and preimplantation embryo. *Mol Reprod Dev* **47**, 148-156 (1997).

61. Chung, C.H. *et al.* Maternal embryonic leucine zipper kinase regulates pancreatic ductal, but not beta-cell, regeneration. *Physiol Rep* **2** (2014).

62. Wang, Y. *et al.* MELK is an oncogenic kinase essential for mitotic progression in basal-like breast cancer cells. *Elife* **3**, e01763 (2014).

63. Gil, M., Yang, Y., Lee, Y., Choi, I. & Ha, H. Cloning and expression of a cDNA encoding a novel protein serine/threonine kinase predominantly expressed in hematopoietic cells. *Gene* **195**, 295-301 (1997).

64. Lin, M.L., Park, J.H., Nishidate, T., Nakamura, Y. & Katagiri, T. Involvement of maternal embryonic leucine zipper kinase (MELK) in mammary carcinogenesis through interaction with Bcl-G, a pro-apoptotic member of the Bcl-2 family. *Breast Cancer Res* **9**, R17 (2007).

65. Nakano, I. *et al.* Maternal embryonic leucine zipper kinase (MELK) regulates multipotent neural progenitor proliferation. *J Cell Biol* **170**, 413-427 (2005).

66. Saito, R., Nakauchi, H. & Watanabe, S. Serine/threonine kinase, Melk, regulates proliferation and glial differentiation of retinal progenitor cells. *Cancer Sci* **103**, 42-49 (2012).

67. Gray, D. *et al.* Maternal embryonic leucine zipper kinase/murine protein serine-threonine kinase 38 is a promising therapeutic target for multiple cancers. *Cancer Res* **65**, 9751-9761 (2005).

68. Kuner, R. *et al.* The maternal embryonic leucine zipper kinase (MELK) is upregulated in high-grade prostate cancer. *J Mol Med (Berl)* **91**, 237-248 (2013).

69. Li, S. *et al.* Maternal embryonic leucine zipper kinase serves as a poor prognosis marker and therapeutic target in gastric cancer. *Oncotarget* **7**, 6266-6280 (2016).

70. Nakano, I. *et al.* Maternal embryonic leucine zipper kinase is a key regulator of the proliferation of malignant brain tumors, including brain tumor stem cells. *J Neurosci Res* **86**, 48-60 (2008).

71. Davezac, N., Baldin, V., Blot, J., Ducommun, B. & Tassan, J.P. Human pEg3 kinase associates with and phosphorylates CDC25B phosphatase: a potential role for pEg3 in cell cycle regulation. *Oncogene* **21**, 7630-7641 (2002).

72. Jung, H., Seong, H.A. & Ha, H. Murine protein serine/threonine kinase 38 activates apoptosis signal-regulating kinase 1 via Thr 838 phosphorylation. *J Biol Chem* **283**, 34541-34553 (2008).

73. Seong, H.A., Gil, M., Kim, K.T., Kim, S.J. & Ha, H. Phosphorylation of a novel zinc-finger-like protein, ZPR9, by murine protein serine/threonine kinase 38 (MPK38). *Biochem J* **361**, 597-604 (2002).

74. Seong, H.A., Kim, K.T. & Ha, H. Enhancement of B-MYB transcriptional activity by ZPR9, a novel zinc finger protein. *J Biol Chem* **278**, 9655-9662 (2003).

75. Wang, Y. *et al.* Mitotic MELK-eIF4B signaling controls protein synthesis and tumor cell survival. *Proc Natl Acad Sci U S A* **113**, 9810-9815 (2016).

76. Jenne, D.E. *et al.* Peutz-Jeghers syndrome is caused by mutations in a novel serine threonine kinase. *Nat Genet* **18**, 38-43 (1998).

77. Mehenni, H. *et al.* Loss of LKB1 kinase activity in Peutz-Jeghers syndrome, and evidence for allelic and locus heterogeneity. *Am J Hum Genet* **63**, 1641-1650 (1998).

78. Deshaies, R.J. Protein degradation: Prime time for PROTACs. *Nat Chem Biol* **11**, 634-635 (2015).

79. Lai, A.C. & Crews, C.M. Induced protein degradation: an emerging drug discovery paradigm. *Nat Rev Drug Discov* **16**, 101-114 (2017).

80. Sakamoto, K.M. *et al.* Protacs: chimeric molecules that target proteins to the Skp1-Cullin-F box complex for ubiquitination and degradation. *Proc Natl Acad Sci U S A* **98**, 8554-8559 (2001).

81. Itoh, Y., Kitaguchi, R., Ishikawa, M., Naito, M. & Hashimoto, Y. Design, synthesis and biological evaluation of nuclear receptor-degradation inducers. *Bioorg Med Chem* **19**, 6768-6778 (2011).

82. Schneekloth, A.R., Pucheault, M., Tae, H.S. & Crews, C.M. Targeted intracellular protein degradation induced by a small molecule: En route to chemical proteomics. *Bioorg Med Chem Lett* **18**, 5904-5908 (2008).

83. Itoh, Y., Ishikawa, M., Naito, M. & Hashimoto, Y. Protein knockdown using methyl bestatin-ligand hybrid molecules: design and synthesis of inducers of ubiquitination-mediated degradation of cellular retinoic acid-binding proteins. *J Am Chem Soc* **132**, 5820-5826 (2010).

84. Orning, L., Krivi, G. & Fitzpatrick, F.A. Leukotriene A4 hydrolase. Inhibition by bestatin and intrinsic aminopeptidase activity establish its functional resemblance to metallohydrolase enzymes. *J Biol Chem* **266**, 1375-1378 (1991).

85. Umezawa, H., Aoyagi, T., Suda, H., Hamada, M. & Takeuchi, T. Bestatin, an inhibitor of aminopeptidase B, produced by actinomycetes. *J Antibiot (Tokyo)* **29**, 97-99 (1976).

86. Sekine, K. *et al.* Small molecules destabilize cIAP1 by activating auto-ubiquitylation. *J Biol Chem* **283**, 8961-8968 (2008).

87. Shibata, N. *et al.* Development of protein degradation inducers of oncogenic BCR-ABL protein by conjugation of ABL kinase inhibitors and IAP ligands. *Cancer Sci* **108**, 1657-1666 (2017).

88. Shibata, N. *et al.* Development of Protein Degradation Inducers of Androgen Receptor by Conjugation of Androgen Receptor Ligands and Inhibitor of Apoptosis Protein Ligands. *J Med Chem* (2017).

89. Gustafson, J.L. *et al.* Small-Molecule-Mediated Degradation of the Androgen Receptor through Hydrophobic Tagging. *Angew Chem Int Ed Engl* **54**, 9659-9662 (2015).

90. Neklesa, T.K. *et al.* Small-molecule hydrophobic tagging-induced degradation of HaloTag fusion proteins. *Nat Chem Biol* **7**, 538-543 (2011).

91. Xie, T. *et al.* Pharmacological targeting of the pseudokinase Her3. *Nat Chem Biol* **10**, 1006-1012 (2014).

92. Long, M.J., Gollapalli, D.R. & Hedstrom, L. Inhibitor mediated protein degradation. *Chem Biol* **19**, 629-637 (2012).

93. Bondeson, D.P. *et al.* Catalytic in vivo protein knockdown by small-molecule PROTACs. *Nat Chem Biol* **11**, 611-617 (2015).

94. Lu, J. *et al.* Hijacking the E3 Ubiquitin Ligase Cereblon to Efficiently Target BRD4. *Chem Biol* **22**, 755-763 (2015).

95. Winter, G.E. *et al.* DRUG DEVELOPMENT. Phthalimide conjugation as a strategy for in vivo target protein degradation. *Science* **348**, 1376-1381 (2015).

96. Zengerle, M., Chan, K.H. & Ciulli, A. Selective Small Molecule Induced Degradation of the BET Bromodomain Protein BRD4. *ACS Chem Biol* **10**, 1770-1777 (2015).

97. Ito, T. *et al.* Identification of a primary target of thalidomide teratogenicity. *Science* **327**, 1345-1350 (2010).

98. Buckley, D.L. *et al.* Small-molecule inhibitors of the interaction between the E3 ligase VHL and HIF1alpha. *Angew Chem Int Ed Engl* **51**, 11463-11467 (2012).

99. Buckley, D.L. *et al.* Targeting the von Hippel-Lindau E3 ubiquitin ligase using small molecules to disrupt the VHL/HIF-1alpha interaction. *J Am Chem Soc* **134**, 4465-4468 (2012).

100. Galdeano, C. *et al.* Structure-guided design and optimization of small molecules targeting the protein-protein interaction between the von Hippel-Lindau (VHL) E3 ubiquitin ligase and the hypoxia inducible factor (HIF) alpha subunit with in vitro nanomolar affinities. *J Med Chem* **57**, 8657-8663 (2014).

101. Gadd, M.S. *et al.* Structural basis of PROTAC cooperative recognition for selective protein degradation. *Nat Chem Biol* **13**, 514-521 (2017).

102. Lai, A.C. *et al.* Modular PROTAC Design for the Degradation of Oncogenic BCR-ABL. *Angew Chem Int Ed Engl* **55**, 807-810 (2016).