Critical Reviews™ in Oncogenesis, 17(2), 233–246 (2012)

Targeting the Human Kinome for Cancer Therapy: Current Perspectives

Luxi Zhang[1] & Roger J. Daly[1,2]*

[1]Cancer Research Program, Garvan Institute of Medical Research; [2]St Vincent's Hospital Clinical School, Department of Medicine, University of New South Wales

*Address all correspondence to: Roger J. Daly, Cancer Research Program, Garvan Institute of Medical Research, 384 Victoria Street, Sydney NSW 2010, Australia; Tel: 61-2-9295-8333; Fax: 61-2-9295-8321; Email: r.daly@garvan.org.au

ABSTRACT: As highlighted by other articles in this review issue, great progress has been made in the development of targeted treatment modalities directed against particular oncogenic kinases, and the translation of these therapies into the clinic. However, recent data from cancer genome sequencing projects indicate that the spectrum of kinases that contribute to cancer progression is wider than previously imagined, and as international projects in this area gather momentum, it appears likely that further kinase oncogenes will be identified, and the roles of previously characterized ones will be extended to subsets of additional cancers. In addition, complementary approaches such as functional genomics and mass spectrometry (MS)-based proteomics are providing important insights into the functional roles played by specific kinases in particular cancers, the dependency of these roles on genetic background, how altered kinase regulation perturbs intracellular signaling networks, and how the latter respond to targeted agents that target the kinase in question. While other articles in this issue focus on individual cancer-associated kinases and their therapeutic targeting, the aim of this review is to take a broader perspective regarding our current knowledge of the cancer kinome and how this can be expanded and exploited for clinical utility.

KEY WORDS: protein kinases, targeted therapy, biomarkers

ABBREVIATIONS

AGC: containing protein kinase A, G, and C; **AML:** acute myeloid leukemia; **CAMK:** calcium/calmodulin-dependent protein kinase; **CMGC:** containing cyclin-dependent kinase, mitogen-activated protein kinase, glycogen synthase kinase 3, and CDC2-like; **CML:** chronic myeloid leukemia; **CK1:** casein kinase 1; **EGFR:** epidermal growth factor receptor; **FDA:** Food and Drug Administration; **FGFR:** fibroblast growth factor receptor; **GIST:** gastrointestinal stromal tumor; **HK2:** hexokinase 2; **LC-MS/MS:** liquid chromatography-tandem mass spectrometry; **mAb:** monoclonal antibody; **MS:** mass spectrometry; **NSCLC:** non-small-cell lung cancer; **PDGFR:** platelet-derived growth factor receptor; **PI3K:** phosphatidylinositol 3-kinase; **RGC:** receptor guanylate cyclase; **RTK:** receptor tyrosine kinase; **SILAC:** stable isotope labeling by amino acids in culture; **SK1:** sphingosine kinase 1; **SFK:** Src family kinase; **STE:** homologs of yeast sterile 7, sterile 11, and sterile 20; **TK:** tyrosine kinase; **TKI:** tyrosine kinase inhibitor; **TKL:** tyrosine kinase-like; **VEGF:** vascular endothelial growth factor.

I. INTRODUCTION

Kinases act as phosphotransferases, transferring phosphate from a high-energy donor molecule, usually ATP, to specific substrates that include proteins, lipids, and carbohydrates, and in doing so act as key regulators of fundamental cellular processes such as growth factor signaling, intracellular trafficking, and metabolism. The human genome encodes 518 protein kinases,[1] categorized into 10 broad groups: AGC (containing protein kinase A, G, and C); CAMK (calcium/calmodulin-dependent protein kinase); CMGC (containing cyclin-dependent kinase, mitogen-activated protein kinase, glycogen synthase kinase 3, and CDC2-like); RGC (receptor guanylate cyclase); TK (tyrosine kinase); TKL (tyrosine kinase–like); STE (homologs of yeast sterile 7, sterile 11, and sterile 20); CK1 (casein kinase 1); atypical; and "other." It also encodes 152 non-protein kinases[2] that are relatively diverse in structure and function and include, for example, 3 families of phosphoinositide lipid kinases. Protein kinases, in particular, are strongly implicated in human disease. For example, approximately one third of all protein kinase genes

0893-9675/12/$35.00

map to cancer-associated amplicons,[1] and this gene family comprises the most common class of mutated genes causally implicated in cancer development.[3] In this article we assess the untapped potential of the human protein and non-protein kinomes in terms of novel targets for cancer therapy, and the approaches that are being used to identify these opportunities.

II. MINING THE CANCER KINOME FOR THERAPEUTIC TARGETS

A. The Mutational Landscape of the Human Kinome

A relatively small number of human cancers are driven by a single, dominant kinase oncogene. Clear examples are chronic myeloid leukemia (CML), where the BCR-ABL oncoprotein is present in 95 % of cases,[4,5] and gastrointestinal stromal tumors (GIST), where activating mutations in the receptor tyrosine kinase KIT are present in up to 85% of patients.[6] Both malignancies often exhibit impressive clinical responses to imatinib, a kinase inhibitor that targets both ABL and KIT.[4,6] Certain other cancers are more heterogenous in terms of their suite of oncogenic changes, but dysregulated signaling by a particular kinase occurs in a significant proportion of patients, due to either mutation or other types of genomic alteration such as amplification or translocation. Examples of this class are: non-small-cell lung cancer (NSCLC), where activating mutations in the epidermal growth factor receptor (EGFR) occur in 15%–30% of patients;[7,8] melanoma, which features mutations in BRAF in approximately 50% of patients;[9,10] and breast cancer, where ERBB2 amplification occurs in approximately 25% of cases.[11,12] Significant clinical responses to targeted therapy (i.e., erlotinib, PLX4032 and trastuzumab, respectively) can also be achieved in these cancers if the responsive subgroup is selected based on the presence of the oncogenic lesion. In addition, activating mutations in FLT3 occur in 30% of acute myeloid leukemia (AML) patients,[13,14] and mutations in PIK3CA, encoding the catalytic subunit of phosphatidylinositol 3-kinase (PI3K), are detected at a similar high frequency in breast, colon, and endometrial cancers.[15,16] Small-molecule inhibitors targeted against these two kinases are currently in clinical development.[14,16] Consequently, when one views the landscape of mutated or dysregulated kinases in human cancer, there are clear "mountains" that stand out on the skyline, and excellent progress has been made in the development and clinical application of therapeutic agents directed against these targets.

However, despite these data, a relatively small fraction of the kinome is currently being targeted, or evaluated, for cancer therapy. If the anti-vascular endothelial growth factor (VEGF)-A mAb bevacizumab is included, then 18 targeted therapies aimed at blocking protein kinase signaling in cancer are currently FDA approved.[17] These are directed against a relatively small number of protein kinases and their oncogenic counterparts, including ABL, SRC, platelet-derived growth factor receptor (PDGFR), KIT, BRAF, ALK, EGFR, ERBB2, mTOR, and the VEGF receptor (VEGFR) family (Fig. 1). In addition, the number of protein kinases currently being targeted in clinical trials represents approximately 10% of this protein family (Fig. 1).[18–20] Notably, the relatively narrow focus of current kinase drug discovery efforts is mirrored by that of academic kinase research,[19] suggesting that there is a general tendency to build upon existing knowledge rather than diverge from the well-beaten track. As discussed below, this engrained behavior has left approximately half of the known protein kinases largely uncharacterized, despite data from either cancer genome sequencing and/or functional genomic approaches that implicate a broad and diverse array of these enzymes in cancer development and cancer cell viability.

Recent cancer genome sequencing initiatives have provided novel and important insights into the distribution of cancer-associated mutations across the protein kinase superfamily. For example, mutation analysis of all protein kinases in 210 specimens derived from 10 common human cancers identified potential "driver" mutations in approximately 120 protein kinases, these occurring in about one-third of the cancer specimens studied (Fig. 1).[21] In addition, a survey of five common cancers for mutations in "candidate" genes that were either implicated in cancer development or encode "druggable" targets

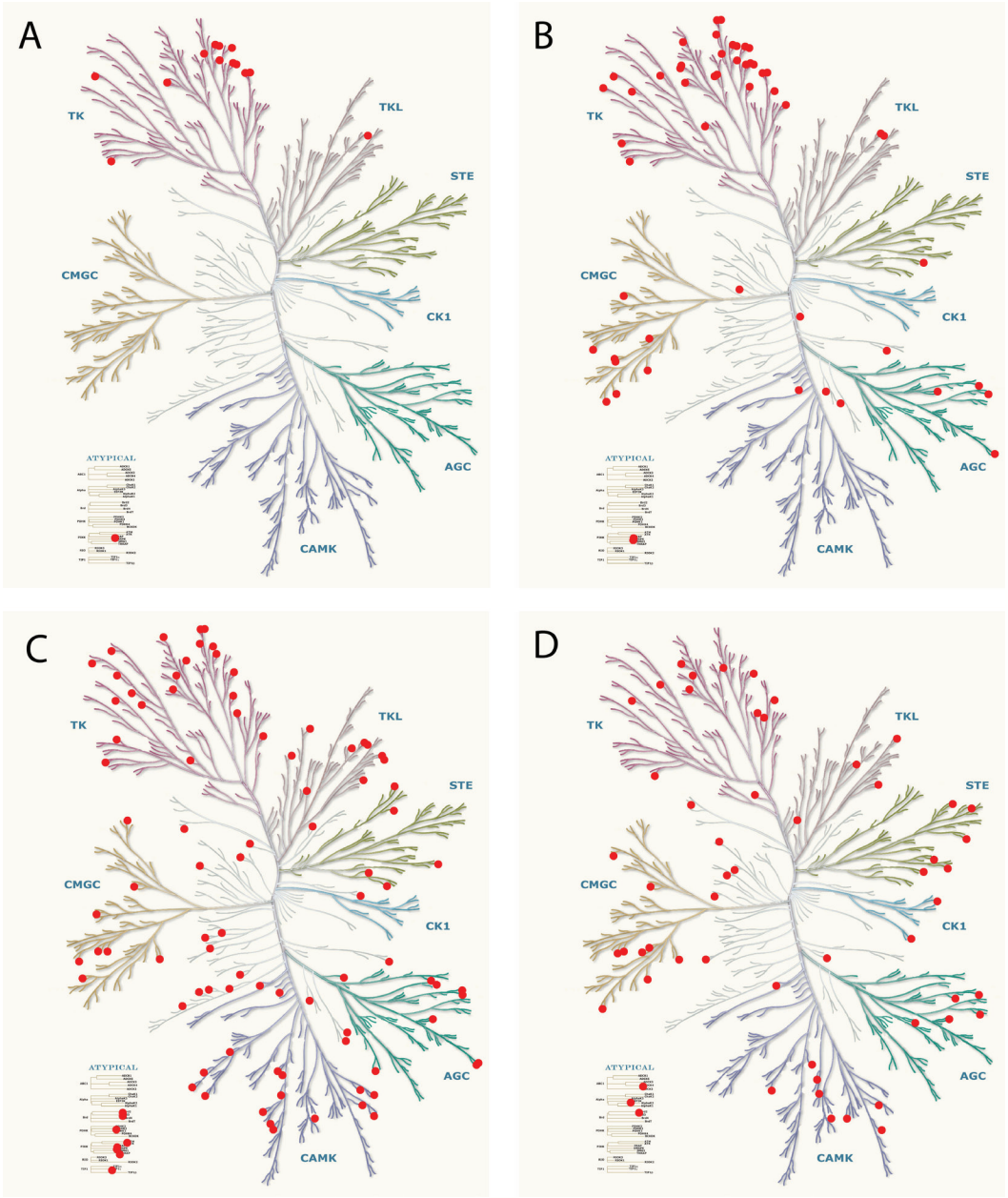

FIGURE 1. The untapped potential of the human kinome. A. Human protein kinases targeted by FDA-approved therapies.[17] Targeted kinases are highlighted by a red circle. B. Targets of kinase inhibitors that are currently in clinical trials in cancer patients.[18–20] C. Kinases that are mutated in common human cancers. The top 100 kinases ordered by conditional probability of carrying at least one driver mutation are highlighted.[21] D. Essential kinases in HeLa cells, as determined by a shRNA screen.[38] Note how the current focus on targeted therapies directed against tyrosine kinases (A and B) contrasts with the number and broad distribution of mutated kinases (C) and essential kinases (D). Kinome tree reproduced courtesy of Cell Signaling Technology, Inc.

identified mutations in 157 of the 230 protein kinases studied.[22] Consequently, these studies indicate that at least 25% of all protein kinases may play a role in oncogenesis. Aside from this revelation, what are the other take-home messages from these studies? First, they indicate that certain kinase families may play a greater role in human malignancy then perhaps envisaged. For example, mutations were detected in 11 different EPH receptors, including EPHB1 and EPHA6.[21,22] Second, many mutations were found outside of the protein kinase families usually associated with cancer development, such as the AGC and TK groups. As an indication, the selection pressure for mutations was highest in the CAMK, TKL, and atypical/other groups of kinases.[21] Examples of poorly characterized kinases that are mutated in a significant proportion of human cancers include DCAMKL1/DCLK1, which is mutated in 15% of gastric cancers, and LRRK2, which is mutated in 8% of multiple myelomas and 11% of ovarian cancers.[21] Third, the mutated kinases may function in processes not classically linked to cancer development. Thus, TAF1L is a component of the TFIID basal transcription factor complex, while ERN1 functions in endoplasmic reticulum stress signaling. Finally, while some mutations, for example within the extracellular domain of FGFRs, are known or are predicted to be activating based on previous research, a significant number of mutations are likely to inactivate the kinase concerned. In this context, loss-of-function mutations in ATM, which functions in DNA damage signaling, and TGFBR2 and BMPR1A, which are receptors for TGFβ superfamily ligands, might be expected due to the nature of the pathways in which these kinases reside. However, likely loss-of-function mutations occur in other kinases, such as DAPK3, HCK, and LYN, all of which exhibit a substitution in the critical aspartate residue of the DFG motif critical for catalysis. In the case of DAPK3, the negative effect of this mutation on kinase activity has been confirmed, and the mutant kinase acts to dominantly inhibit the function of the wild-type kinase,[23] identifying DAPK3 as a tumor suppressor kinase. Similarly, several cancer-associated mutations in MAP2K4, which functions in the JNK pathway, reduce kinase activity, and expression of these mutants promotes anchorage-independent growth.[22] However, this situation is complicated by recent findings regarding BRAF, where D594 substitutions in the DFG motif, the third most common B-Raf mutation in human cancer, can promote signaling to MEK and ERK in the presence of activated K-Ras. This occurs due to heterodimerization of BRAF with CRAF and activation of the latter kinase.[24] These findings highlight the need to carefully characterize the functional effects of individual kinase mutations, which may be context dependent.

B. Identification of Oncogenic Protein Kinases by Mass Spectrometry–Based Phosphoproteomics

An alternative strategy for detecting aberrantly activated kinases in cancer cells is through the application of mass spectrometry (MS)-based proteomic approaches in combination with affinity-based purification techniques that enrich particular subproteomes, and/or quantification strategies such as stable isotope labeling by amino acids in culture (SILAC).[25] Such strategies provide important information not provided by cancer genome sequencing, such as the identification of kinases dysregulated due to overexpression rather than mutation, the context of aberrant kinase signaling (such as the presence of other, potentially cooperating kinases), and the global impact of an oncogenically activated kinase on a particular type of signaling event within the cell (such as tyrosine phosphorylation).

One powerful strategy is the use of anti-phosphotyrosine antibodies to purify tyrosine-phosphorylated peptides from proteolytically digested lysates derived from cancer cell lines or tumors, followed by peptide identification and quantification by liquid chromatography–tandem MS (LC-MS/MS). This approach led to the identification of TEL-ARG, BCR-ABL, and FGFR1OP2-FGFR1 fusion oncoproteins, and a JAK2 pseudokinase domain mutation, in 4 AML cell lines,[26,27] and a novel JAK2 mutation in an acute megakaryoblastic leukemia cell line.[28] Extension of this approach to a large panel of NSCLC tumors and cell lines identified aberrant activation of several novel driver kinases in this malignancy,

including ALK and ROS fusion proteins, DDR1, and PDGFRα.[29] Interestingly, the approach also resolved subgroups of NSCLCs characterized by distinct patterns of activated tyrosine kinases that might be expected to exhibit contrasting sensitivity to particular TK-directed therapies. More recently, tyrosine phosphorylation profiling of a wide panel of breast cancer cell lines revealed that the basal subgroup is characterized by a prominent Src family kinase (SFK) signaling network, and identified several tyrosine kinases exhibiting marked phosphorylation in this subgroup, including EGFR, MET, EPHA2, and LYN.[30] This suggests that effective therapeutic targeting of basal breast cancers may necessitate the use of multi-kinase inhibitors or combination therapies. A further application of this strategy is the characterization of kinase-mediated bypass mechanisms that lead to resistance to targeted therapies. For example, the approach has been used to identify an important role for SFKs in mediating lapatinib resistance in HER2-overexpressing breast cancer cells,[31] as well as SYK and LYN in promoting nilotinib resistance in CML.[32]

Application of alternative purification methodologies allows other kinase-linked sub-proteomes to be interrogated by MS. As described elsewhere in this issue,[16] PI3K is commonly activated in human cancers, and this can occur due to genetic changes in the cancer, such as PIK3CA mutation or PTEN loss, or coupling to particular tyrosine kinases.[16] Identification of these kinases may lead to improved strategies for therapeutic targeting. In a recent study, Yang and colleagues used immunoaffinity purification of the p85 subunit of PI3K, in combination with a targeted MS/MS approach, to identify docking proteins and receptor tyrosine kinases (RTKs) associated with PI3K. This successfully identified key PI3K interactors in cancer cell lines and in a xenograft model, and revealed how PI3K complexes were altered by treatment with specific TK inhibitors (TKIs), or rapamycin, which relieves negative feedback regulation of PI3K signaling to Akt.[33] Finally, while the aforementioned approaches focus largely on tyrosine kinases, the use of broad-selectivity kinase inhibitors as capture reagents can be used to extend purification to the significant fraction of the human kinome that is not tyrosine phosphorylated. This had previously presented a technical challenge, because peptide enrichment based solely on protein phosphorylation status leads to an under-representation of the protein kinase subclass in subsequent MS analyses, reflecting the low cellular abundance of many protein kinases.[34] However, coupling of multiple broad-specificity kinase ligands to beads (to create "kinobeads"),[35] or use of a series of affinity columns containing inhibitors with distinct but overlapping selectivity profiles,[34] enables MS-based detection of approximately 200 different protein kinases from cell extracts. Since a given mammalian cell is thought to express approximately 300 different protein kinases,[36] this approach provides coverage of the majority of the expressed kinome. Furthermore, when used in combination with quantitation techniques such as SILAC, it can be used to compare the expressed kinome, in terms of both protein levels and activation status, between different cell types and treatment conditions.[34,37] It should be noted that comparable insights cannot be obtained by antibody-based strategies, since the reagents required to attain this coverage have yet to be generated and validated. Consequently, this represents a powerful strategy for identification of novel oncogenic kinases, particularly if dysregulation is primarily at the post-transcriptional level. In addition, by providing important information such as the phosphorylation status of a novel oncogenic kinase, and how the remainder of the kinome responds to its expression, it is likely to complement other approaches such as cancer genome sequencing.

C. Functional Annotation of the Human Kinome in Cancer Cells

Clearly, the presence of an activating mutation, or marked overexpression, can highlight a driver kinase that is essential for cancer cell proliferation and/or survival. However, an alternative strategy for identifying potential cancer drug targets is to go straight to the heart of the matter and ask which kinases, irrespective of their mutation or expression status, are essential for the viability of a given cancer cell. The development of high-throughput functional si/shRNA screening methodologies has

enabled this question to be addressed, and the results are surprising.

Using a lentiviral shRNA library that targeted ~85% of the kinome, Grueneberg and colleagues screened a variety of cell lines for essential kinases whose knockdown significantly reduced cell proliferation and survival.[38] The main findings from the initial screens, undertaken in HeLa and 293-T cells as well as four NSCLC lines, can be summarized as follows: first, a relatively large number of kinases (on average 50–100) were identified as being essential in any one line (Fig. 1); second, similar to the results of cancer genome sequencing studies, many of the identified kinases were relatively uncharacterized; and third, there was remarkably little overlap between different cell lines in their kinase requirements. To emphasize the latter point, only 5% of the 430 kinases tested were required in all four NSCLC cell lines. The closest kinase requirements were observed for primary cell lines from identical tissue sources, followed by essentially isogenic cancer lines that differed in expression of a single oncoprotein. These findings indicate that due to the myriad of genetic and epigenetic events that occur during cancer development and progression, different cancers arising in a particular tissue type can differ greatly in the composition and wiring of their kinase networks, and hence their suite of essential kinases. While this complicates development of kinase-directed therapeutics for particular malignancies, the discovery that otherwise isogenic lines differing in expression of a single oncogene[39] or tumor suppressor[40] exhibit differential dependency on a discrete subset of kinases presents the opportunity to identify resistance mechanisms for certain targeted therapeutics, as well as the Achilles' heel for cancer cells exhibiting specific genetic alterations. For example, $VHL^{-/-}$ renal carcinoma cells exhibit a greater dependency on CDK6 than their pVHL-reconstituted counterparts and are preferentially inhibited by a small-molecule CDK4/6 inhibitor.[40]

More recently, several groups have used RNAi screens to identify kinases that exhibit "synthetic-lethal" interactions with defined cancer-related genetic alterations, and therefore represent potential genotype-selective therapeutic targets. An additional value of such screens is their ability to identify drug-gable targets for cancers where the driving oncogenic event has proven refractory to therapeutic development. For example, focused RNAi screens identified the serine/threonine kinases TBK1[41] and STK33[42] as being selectively essential in cells expressing mutant KRas, although the role of STK33 has been called into question,[43] and a genome-wide shRNA screen revealed that KRas mutant cells exhibit mitotic stress and exhibit increased sensitivity to PLK1 inhibition.[44] In addition, a recent study determined that primary human epithelial cells from three different tissues exhibit a requirement for either SGK2 or PAK3 following p53 inactivation.[45] Finally, the Ashworth group has extended this approach by integrating data from kinome-wide RNAi screens across a wide breast cancer cell line panel with corresponding genomic, transcriptomic and mutation analyses in order to characterize the kinase dependency of breast cancer subtypes.[46] This identified many candidate genetic dependencies, and two key genetic interactions, the requirement for the mitotic checkpoint kinase TTK in PTEN-deficient cells, and for ADCK2 in estrogen receptor-positive cells, were validated by additional experimentation. An important aspect of this approach is that it identifies candidate synthetic lethal effects that are relatively unaffected by additional genetic changes, and which therefore highlight potential strategies for therapeutic targeting of a significant proportion of a given cancer subtype. Subsequent validation can then be undertaken using near-isogenic pairs of lines differing only in the genetic alteration of interest.

D. Non-Protein Kinases and Cancer

Although this review has largely focused on the protein kinase family, specific members of other kinase categories are also strongly implicated in human cancer. As reviewed elsewhere in this issue, the most striking example is PIK3CA,[16] but other lipid kinases also contribute to cancer progression and some are being pursued as therapeutic targets. For example: high expression of PIP5K1C (which promotes formation of PI4,5P2 from PI4P) in breast cancer is associated with poor prognosis, and knockdown of this enzyme reduces breast cancer cell migration,

invasion, and proliferation;[47] ERBB2-amplified breast cancer cells appear to be sensitized to knockdown of PIP5K1A, compared to non-amplified counterparts;[46] and overexpression of sphingosine kinase 1 (SK1) occurs in a variety of human cancers, leading to major efforts to develop novel therapeutics that target this enzyme, its product S1P, or S1P-specific G protein coupled receptors.[48] Other kinases phosphorylate small-molecule vitamins and metabolites, and particular members of this grouping also have roles in human malignancies. One example is choline kinase α, which displays increased expression in many cancers, and targeting this enzyme with a small-molecule competitive inhibitor of substrate binding attenuated growth of lung cancer cells in a xenograft model.[49] In addition, partly reflecting a recent resurgence in interest in the Warburg effect, whereby cancer cells switch to aerobic glycolysis to promote growth, several kinases that function in the glycolytic pathway are being investigated as potential therapeutic targets. These include the cancer-selective M2 isoform of pyruvate kinase (PKM2),[50] hexokinase 2 (HK2), which is critical for the Warburg effect in glioblastoma multiforme,[51] and the 6-phosphofructo-2-kinase/fructose-2,6-bisphosphatase PFKFB3, which is overexpressed in several cancers and validated as a potential therapeutic target in preclinical models.[52] Since recent high-profile findings regarding the role of particular metabolic enzymes in cancer[53,54] and the dependency of particular cancers on specific metabolic or biosynthetic pathways[55,56] have provided added momentum to the field of cancer cell metabolism, it appears likely that additional small-molecule kinases will be identified as potential cancer drug targets.

III. THERAPEUTIC TARGETING OF THE CANCER KINOME

A. Therapeutic Development

The development of small-molecule,[57,58] antibody-based,[59] and non-immunoglobulin protein scaffolds[60] as cancer therapeutics has been the subject of several excellent reviews, and so only certain broad concepts will be addressed here, with the major focus on small-molecule inhibitors. Two issues deserve emphasis. First, in order to exploit recent findings regarding kinase mutations and dependencies in cancer cells, the number of kinases that can be targeted by highly selective therapeutic agents needs to be greatly expanded. Second, since acquired resistance to existing therapies presents a major clinical problem, additional strategies to counter such resistance mechanisms must be developed. With regard to both issues, several important lessons have been learned from our experience with therapeutic targeting of BCR-ABL in CML. Here, a variety of drugs have been developed that exhibit different binding modes to the ABL kinase domain, and these are used to treat different stages of the disease, depending on the emergence of specific kinase domain mutations. The discovery that the front-line drug imatinib binds to the ABL kinase domain in the inactive "DFG-out" conformation was important because it identified an inhibitor binding mode that is potentially more amenable to the development of kinase-selective agents.[58] Other kinase inhibitors are now known to bind their targets in this manner, such as lapatinib and sorafenib, and are classified as type II inhibitors.[61] Of the two second-generation BCR-ABL inhibitors developed to counter the emergence of imatinib-resistant BCR-ABL mutants, dasatinib binds to the active conformation in the ATP pocket (type I inhibitor), while nilotinib, like imatinib, is type II.[61] However, a particular BCR-ABL mutant, T315I, is resistant to all three inhibitors, and has spurred the development of third-generation TKIs. Two of these highlight other modes of action. Specifically, the "switch-control" inhibitor DCC-2036 induces an inactive conformation of the ABL kinase domain and represents a distinct subclass of type II inhibitor,[62] while GNF-5 is an allosteric, type III inhibitor that binds the myristoyl pocket of ABL and overcomes T315I-mediated resistance if used in combination with imatinib or nilotinib.[63]

Overall, these findings indicate that for certain driver kinases the evolution of drug resistance through target mutation can be repeatedly countered with different inhibitors. In addition, it highlights the multiple types of inhibitor that have been developed, with type II and type III inhibitors in particular holding great promise in terms of attaining high target

selectivity.[64,65] Importantly, these concepts have been applied to other kinases, indicating that they have broader applicability. For example, type III inhibitors have been developed against a range of other kinases, including AKT, CHK1, and PDK1,[58,65] as well as EGFR kinase inhibitors that are selective for the EGFR T790M mutation, which confers resistance to gefitinib and erlotinib.[66] Extension of these concepts across the kinome will require detailed structural information regarding currently uncharacterized kinases, as well as mutant forms of characterized ones, in order to facilitate rational drug design, and a thorough understanding of kinase regulation and function. The latter point is underscored by recent findings regarding the complexity of action of BRAF inhibitors.[10] In addition, it is likely that new scaffolds will need to be developed in order to hit new targets while maintaining target selectivity,[57,67] although recent large-scale profiling studies of kinase inhibitor selectivity provide lead chemotypes for certain orphan kinases, such as haspin.[64,68]

Although some cancers, such as CML, can be effectively treated by inhibiting a single oncogenic kinase, it is becoming increasingly evident that other cancers will require targeting of multiple kinases. This may reflect, for example, the concomitant activation of multiple receptor tyrosine kinases, as observed in basal breast cancer[30] and glioblastoma,[69] or mutation of a downstream signaling molecule that is itself a kinase (e.g., PI3K) or couples to kinase effectors (e.g., KRas).[8,10,12,70] In addition, kinase crosstalk or negative feedback loops may lead to activation of other kinases following inhibitor treatment, and resistance to specific kinase inhibitors may arise via kinase bypass mechanisms.[8,70] Inhibition of multiple kinases can be achieved via combining two or more therapeutic agents, using multikinase inhibitors such as sorafenib (therapeutically relevant targets: VEGFR2 and PDGFR), sunitinib (VEGFR2, PDGFR, KIT), and vandetanib (VEGFR, EGFR, RET), or using bispecific antibodies (examples include HER2/VEGF- and HER2/HER3-specific immunotherapeutics).[70–73] Therapeutic strategies that target multiple kinase pathways and are currently in clinical trials include the use of various mTOR inhibitors in combination with antibodies or inhibitors that target signaling by specific RTKs (e.g., bevacizumab, trastuzumab), MK2206 (AKT) in combination with AZD6244 (MEK), and various multikinase inhibitors.[16,70,71] Further characterization of signaling networks associated with particular cancers, and how these are perturbed by kinase-directed therapeutics, is likely to identify additional settings where effective treatment necessitates targeting multiple kinases. Such advances will be complemented by burgeoning interest in the rational design of multitargeted drugs, or targeted polypharmacology.[71] Recent advances in this area include the generation of a kinome interaction network based on both sequence information and kinase inhibitor profiling data, which enables the pharmacological relationships between two kinases to be interrogated in the context of particular chemotypes,[74] and comprehensive analyses of kinase inhibitor selectivity across the kinome that have identified shared chemotype preferences between unrelated kinases.[64, 68]

B. Patient Stratification and Predictive Biomarkers

The importance of matching targeted therapy with the appropriate patient population is highlighted by prior experience with trastuzumab, where its efficacy against HER2-amplified/overexpressing breast cancers may have gone undetected if the trial had utilized an unselected patient population,[75] and with EGFR kinase inhibitors in NSCLC, where the utility of these drugs in the clinical setting was not clarified until EGFR mutations were detected in the subset of patients that respond to these drugs.[7] As a consequence, it is now widely accepted that companion biomarkers should be developed in parallel with novel therapeutic agents. In the context of kinase-directed therapies, such markers usually represent the presence of mutation or amplification of the target. However, these may not always correlate tightly with sensitivity to target inhibition, as found with PIK3CA mutations.[16] Consequently, additional markers that associate positively or negatively with response may need to be identified, for example, via phosphoproteomic analysis of the kinase-regulated pathway,[76] or detailed characterization of signaling by

the target kinase, as exemplified by recent research on BRAF.[10,24,77,78] An excellent example of a predictive biomarker that has entered clinical use is the presence of mutant KRas in colorectal cancer, which is associated with lack of response to cetuximab.[8] Of note, while a coordinated effort towards predictive biomarker identification and validation is necessary for optimal translation of new therapies into the clinic, discovery and refinement of companion biomarkers for existing targeted agents will improve their efficacy and may also identify novel responsive patient subgroups.

It appears likely that further characterization of cancer kinomes will lead to a significant expansion of our armory of candidate targeted therapeutics, each directed against a particular molecular phenotype. While this is an exciting prospect, rapid exploitation of these advances for patient benefit will necessitate major changes to the drug development pipeline and novel clinical trial design. One potential strategy will be to implement more rational early-phase clinical trial designs, so that phase I trials not only determine drug dosage but also attempt to identify patient subpopulations that respond, leading to more efficient phase I–III development strategies.[79,80] In addition, adaptive-type clinical trials will provide the capability to respond to evolving information regarding predictive biomarker/targeted therapy relationships as the trial is running. An example of this is the BATTLE adaptive phase II trial in advanced NSCLC, where following patient recruitment, fresh core needle biopsy specimens were taken and assayed for four biomarkers mechanistically related to targeted therapies involved in the trial.[81] A subset of the patient cohort (38%) was initially randomized equally to one of four treatments. Following assessment of disease control rate, randomization probabilities for the remaining patients were adjusted in the subsequent adaptive phase using a Bayesian model, so that patients with a particular biomarker profile had a greater than 25% chance of being assigned to a treatment that appeared effective, based on previous disease control rates for patients with the same signature. Among the biomarker/treatment relationships reported in this study were positive associations between EGFR mutations or VEGF/VEGFR2 expression and erlotinib response, as well as between *KRAS* or *BRAF* mutation positivity and sorafenib response. Interestingly, EGFR mutation or polysomy was associated with a poor response to sorafenib. Finally, since a particular molecular phenotype (e.g., activating mutation in a given kinase) may occur in cancers from multiple organs, but at a low frequency in each, a "biotype" classification of cancer where malignancies are grouped together based on molecular phenotypes rather than organ of origin or morphology may be necessary to enable recruitment of sufficient patients into clinical trials of an appropriate targeted therapeutic.[82] Overall, it is anticipated that these novel approaches to clinical trial design will facilitate the rapid evaluation of novel targeted treatments and companion biomarkers, and hence expedite progress towards personalized cancer therapy.

IV. CONCLUSION

Recent advances in our knowledge of the cancer kinome and strategies for kinase-directed drug development place us in a strong position to build on previous achievements regarding therapeutic targeting of oncogenic kinases. However, past experiences have taught us that development of clinically effective targeted therapies requires a detailed understanding of target mechanism and function, its network role, the mode of action of the therapy and its impact on intracellular signaling, the elucidation of resistance mechanisms, identification of companion biomarkers, and effective patient stratification for therapy. In light of these considerations, it is increasingly evident that integrative research approaches, as well as extensive cross-disciplinary collaboration between scientists and clinicians, will be necessary to efficiently deliver positive clinical outcomes.

ACKNOWLEDGMENTS

Research in RJD's laboratory is funded by the National Health and Medical Research Council of Australia, Cancer Council NSW, and Cancer Institute NSW. LZ is the recipient of an Australian Postgraduate Award.

REFERENCES

1. Manning G, Whyte DB, Martinez R, Hunter T, Sudarsanam S. The protein kinase complement of the human genome. Science. 2002;298(5600): 1912–1934.
2. Park J, Hu Y, Murthy TV, Vannberg F, Shen B, Rolfs A, Hutti JE, Cantley LC, Labaer J, Harlow E, Brizuela L. Building a human kinase gene repository: bioinformatics, molecular cloning, and functional validation. Proc Natl Acad Sci U S A. 2005;102(23):8114–19.
3. Futreal PA, Coin L, Marshall M, Down T, Hubbard T, Wooster R, Rahman N, Stratton MR. A census of human cancer genes. Nat Rev Cancer. 2004;4(3):177–83.
4. Sullivan C, Peng C, Chen Y, Li D, Li S. Targeted therapy of chronic myeloid leukemia. Biochem Pharmacol. 2010;80(5):584–91.
5. Yeung DT, Hughes TP. Therapeutic targeting of Bcr-Abl: prognostic markers of response and resistance mechanism in chronic myeloid leukaemia. Crit Rev Oncogenesis. This issue.
6. Antonescu CR. The GIST paradigm: lessons for other kinase-driven cancers. J Pathol. 2011;223(2):251–61.
7. Pao W, Chmielecki J. Rational, biologically based treatment of EGFR-mutant non-small-cell lung cancer. Nat Rev Cancer. 2010;10(11):760–74.
8. Dhomen NS, Mariadason J, Tebbutt N, Scott AM. Therapeutic targeting of the epidermal growth factor receptor in human cancer. Crit Rev Oncogenesis. This issue.
9. Flaherty KT, Fisher DE. New strategies in metastatic melanoma: oncogene-defined taxonomy leads to therapeutic advances. Clin Cancer Res. 2011;17(15):4922–28.
10. Roring M, Brummer T. Aberrant B-Raf signalling in human cancer—10 years from bench to bedside. Crit Rev Oncogenesis. This issue.
11. Hudis CA. Trastuzumab—mechanism of action and use in clinical practice. N Engl J Med. 2007;357(1):39–51.
12. Rexer BN, Arteaga CL. Intrinsic and acquired resistance to HER2-targeted therapies in HER2 gene-amplified breast cancer: mechanisms and clinical implications. Crit Rev Oncogenesis. This issue.
13. Pemmaraju N, Kantarjian H, Ravandi F, Cortes J. FLT3 inhibitors in the treatment of acute myeloid leukemia: the start of an era? Cancer. 2011;117(15):3293–304.
14. Langdon WY. FLT3 signaling and the development of inhibitors that target FLT3 kinase activity. Crit Rev Oncogenesis. This issue.
15. Samuels Y, Waldman T. Oncogenic mutations of PIK3CA in human cancers. Curr Top Microbiol Immunol. 2010;347:21–41.
16. Sheppard KE, Kinross KM, Solomon B, Pearson RB, Phillips WA. Targeting PI3Kinase/AKT/MTOR signaling in cancer. Crit Rev Oncogenesis. This issue.
17. FactSheet available from http://www.cancer.gov/.
18. Matthews DJ, Gerritsen ME. Targeting protein kinases for cancer therapy. Hoboken, New Jersey: John Wiley & Sons Inc.; 2010.
19. Fedorov O, Muller S, Knapp S. The (un)targeted cancer kinome. Nat Chem Biol. 2010;6(3):166–69.
20. Information from http://clinicaltrials.gov/.
21. Greenman C, Stephens P, Smith R, Dalgliesh GL, Hunter C, Bignell G, Davies H, Teague J, Butler A, Stevens C, Edkins S, O'Meara S, Vastrik I, Schmidt EE, Avis T, Barthorpe S, Bhamra G, Buck G, Choudhury B, Clements J, Cole J, Dicks E, Forbes S, Gray K, Halliday K, Harrison R, Hills K, Hinton J, Jenkinson A, Jones D, Menzies A, Mironenko T, Perry J, Raine K, Richardson D, Shepherd R, Small A, Tofts C, Varian J, Webb T, West S, Widaa S, Yates A, Cahill DP, Louis DN, Goldstraw P, Nicholson AG, Brasseur F, Looijenga L, Weber BL, Chiew YE, DeFazio A, Greaves MF, Green AR, Campbell P, Birney E, Easton DF, Chenevix-Trench G, Tan MH, Khoo SK, Teh BT, Yuen ST, Leung SY, Wooster R, Futreal PA, Stratton MR. Patterns of somatic mutation in human cancer genomes. Nature. 2007;446(7132):153–58.
22. Kan Z, Jaiswal BS, Stinson J, Janakiraman V, Bhatt D, Stern HM, Yue P, Haverty PM, Bourgon R, Zheng J, Moorhead M, Chaudhuri S, Tomsho LP, Peters BA, Pujara K, Cordes S, Davis DP, Carlton VE, Yuan W, Li L, Wang W, Eigenbrot C, Kaminker JS, Eberhard DA, Waring P, Schuster SC, Modrusan Z, Zhang Z, Stokoe D, de Sauvage FJ, Faham M, Seshagiri S. Diverse somatic mutation patterns and pathway alterations in human cancers. Nature. 2010;466(7308):869–73.
23. Brognard J, Zhang YW, Puto LA, Hunter T. Cancer-associated loss-of-function mutations implicate DAPK3 as a tumor-suppressing kinase. Cancer Res. 2011;71(8):3152–61.
24. Heidorn SJ, Milagre C, Whittaker S, Nourry A, Niculescu-Duvas I, Dhomen N, Hussain J, Reis-Filho JS, Springer CJ, Pritchard C, Marais R.

Kinase-dead BRAF and oncogenic RAS cooperate to drive tumor progression through CRAF. Cell. 2010;140(2):209–21.
25. Macek B, Mann M, Olsen JV. Global and site-specific quantitative phosphoproteomics: principles and applications. Annu Rev Pharmacol Toxicol. 2009;49:199–221.
26. Walters DK, Goss VL, Stoffregen EP, Gu TL, Lee K, Nardone J, McGreevey L, Heinrich MC, Deininger MW, Polakiewicz R, Druker BJ. Phosphoproteomic analysis of AML cell lines identifies leukemic oncogenes. Leuk Res. 2006;30(9):1097–104.
27. Gu TL, Goss VL, Reeves C, Popova L, Nardone J, Macneill J, Walters DK, Wang Y, Rush J, Comb MJ, Druker BJ, Polakiewicz RD. Phosphotyrosine profiling identifies the KG-1 cell line as a model for the study of FGFR1 fusions in acute myeloid leukemia. Blood. 2006;108(13):4202–4.
28. Mercher T, Wernig G, Moore SA, Levine RL, Gu TL, Frohling S, Cullen D, Polakiewicz RD, Bernard OA, Boggon TJ, Lee BH, Gilliland DG. JAK2T875N is a novel activating mutation that results in myeloproliferative disease with features of megakaryoblastic leukemia in a murine bone marrow transplantation model. Blood. 2006;108(8):2770–79.
29. Rikova K, Guo A, Zeng Q, Possemato A, Yu J, Haack H, Nardone J, Lee K, Reeves C, Li Y, Hu Y, Tan Z, Stokes M, Sullivan L, Mitchell J, Wetzel R, Macneill J, Ren JM, Yuan J, Bakalarski CE, Villen J, Kornhauser JM, Smith B, Li D, Zhou X, Gygi SP, Gu TL, Polakiewicz RD, Rush J, Comb MJ. Global survey of phosphotyrosine signaling identifies oncogenic kinases in lung cancer. Cell. 2007;131(6):1190–203.
30. Hochgrafe F, Zhang L, O'Toole SA, Browne BC, Pinese M, Porta Cubas A, Lehrbach GM, Croucher DR, Rickwood D, Boulghourjian A, Shearer R, Nair R, Swarbrick A, Faratian D, Mullen P, Harrison DJ, Biankin AV, Sutherland RL, Raftery MJ, Daly RJ. Tyrosine phosphorylation profiling reveals the signaling network characteristics of basal breast cancer cells. Cancer Res. 2010;70(22):9391–401.
31. Rexer BN, Ham AJ, Rinehart C, Hill S, Granja-Ingram Nde M, Gonzalez-Angulo AM, Mills GB, Dave B, Chang JC, Liebler DC, Arteaga CL. Phosphoproteomic mass spectrometry profiling links Src family kinases to escape from HER2 tyrosine kinase inhibition. Oncogene. 2011;30(40):4163–74.
32. Gioia R, Leroy C, Drullion C, Lagarde V, Etienne G, Dulucq S, Lippert E, Roche S, Mahon FX, Pasquet JM. Quantitative phosphoproteomics revealed interplay between Syk and Lyn in the resistance to nilotinib in chronic myeloid leukemia cells. Blood. 2011;118(8):2211–21.
33. Yang X, Turke AB, Qi J, Song Y, Rexer BN, Miller TW, Janne PA, Arteaga CL, Cantley LC, Engelman JA, Asara JM. Using tandem mass spectrometry in targeted mode to identify activators of class IA PI3K in cancer. Cancer Res. 2011;71(18):5965–75.
34. Daub H, Olsen JV, Bairlein M, Gnad F, Oppermann FS, Korner R, Greff Z, Keri G, Stemmann O, Mann M. Kinase-selective enrichment enables quantitative phosphoproteomics of the kinome across the cell cycle. Mol Cell. 2008;31(3):438–48.
35. Bantscheff M, Eberhard D, Abraham Y, Bastuck S, Boesche M, Hobson S, Mathieson T, Perrin J, Raida M, Rau C, Reader V, Sweetman G, Bauer A, Bouwmeester T, Hopf C, Kruse U, Neubauer G, Ramsden N, Rick J, Kuster B, Drewes G. Quantitative chemical proteomics reveals mechanisms of action of clinical ABL kinase inhibitors. Nat Biotechnol. 2007;25(9):1035–44.
36. Su AI, Cooke MP, Ching KA, Hakak Y, Walker JR, Wiltshire T, Orth AP, Vega RG, Sapinoso LM, Moqrich A, Patapoutian A, Hampton GM, Schultz PG, Hogenesch JB. Large-scale analysis of the human and mouse transcriptomes. Proc Natl Acad Sci U S A. 2002;99(7):4465–70.
37. Oppermann FS, Gnad F, Olsen JV, Hornberger R, Greff Z, Keri G, Mann M, Daub H. Large-scale proteomics analysis of the human kinome. Mol Cell Proteomics. 2009;8(7):1751–64.
38. Grueneberg DA, Degot S, Pearlberg J, Li W, Davies JE, Baldwin A, Endege W, Doench J, Sawyer J, Hu Y, Boyce F, Xian J, Munger K, Harlow E. Kinase requirements in human cells: I. Comparing kinase requirements across various cell types. Proc Natl Acad Sci U S A. 2008;105(43):16472–77.
39. Baldwin A, Li W, Grace M, Pearlberg J, Harlow E, Munger K, Grueneberg DA. Kinase requirements in human cells: II. Genetic interaction screens identify kinase requirements following HPV16 E7 expression in cancer cells. Proc Natl Acad Sci U S A. 2008;105(43):16478–83.
40. Bommi-Reddy A, Almeciga I, Sawyer J, Geisen C, Li W, Harlow E, Kaelin WG, Jr., Grueneberg DA. Kinase requirements in human cells: III. Altered kinase requirements in VHL-/- cancer cells detected in a pilot synthetic lethal screen. Proc Natl Acad Sci U S A. 2008;105(43):16484–89.

41. Barbie DA, Tamayo P, Boehm JS, Kim SY, Moody SE, Dunn IF, Schinzel AC, Sandy P, Meylan E, Scholl C, Frohling S, Chan EM, Sos ML, Michel K, Mermel C, Silver SJ, Weir BA, Reiling JH, Sheng Q, Gupta PB, Wadlow RC, Le H, Hoersch S, Wittner BS, Ramaswamy S, Livingston DM, Sabatini DM, Meyerson M, Thomas RK, Lander ES, Mesirov JP, Root DE, Gilliland DG, Jacks T, Hahn WC. Systematic RNA interference reveals that oncogenic KRAS-driven cancers require TBK1. Nature. 2009;462(7269):108–12.
42. Scholl C, Frohling S, Dunn IF, Schinzel AC, Barbie DA, Kim SY, Silver SJ, Tamayo P, Wadlow RC, Ramaswamy S, Dohner K, Bullinger L, Sandy P, Boehm JS, Root DE, Jacks T, Hahn WC, Gilliland DG. Synthetic lethal interaction between oncogenic KRAS dependency and STK33 suppression in human cancer cells. Cell. 2009;137(5):821–34.
43. Babij C, Zhang Y, Kurzeja RJ, Munzli A, Shehabeldin A, Fernando M, Quon K, Kassner PD, Ruefli-Brasse AA, Watson VJ, Fajardo F, Jackson A, Zondlo J, Sun Y, Ellison AR, Plewa CA, San MT, Robinson J, McCarter J, Schwandner R, Judd T, Carnahan J, Dussault I. STK33 kinase activity is nonessential in KRAS-dependent cancer cells. Cancer Res. 2011;71(17):5818–26.
44. Luo J, Emanuele MJ, Li D, Creighton CJ, Schlabach MR, Westbrook TF, Wong KK, Elledge SJ. A genome-wide RNAi screen identifies multiple synthetic lethal interactions with the Ras oncogene. Cell. 2009;137(5):835–48.
45. Baldwin A, Grueneberg DA, Hellner K, Sawyer J, Grace M, Li W, Harlow E, Munger K. Kinase requirements in human cells: V. Synthetic lethal interactions between p53 and the protein kinases SGK2 and PAK3. Proc Natl Acad Sci U S A. 2010;107(28):12463–68.
46. Brough R, Frankum JR, Sims D, Mackay A, Mendes-Pereira AM, Bajrami I, Costa-Cabral S, Rafiq R, Ahmad AS, Cerone MA, Natrajan R, Sharpe R, Shiu KK, Wetterskog D, Dedes KJ, Lambros MB, Rawjee T, Linardopoulos S, Reis-Filho JS, Turner NC, Lord CJ, Ashworth A. Functional viability profiles of breast cancer. Cancer Discov. 2011;1(3):260–73.
47. Sun Y, Turbin DA, Ling K, Thapa N, Leung S, Huntsman DG, Anderson RA. Type I gamma phosphatidylinositol phosphate kinase modulates invasion and proliferation and its expression correlates with poor prognosis in breast cancer. Breast Cancer Res. 2010;12(1):R6.
48. Pyne S, Bittman R, Pyne NJ. Sphingosine kinase inhibitors and cancer: seeking the golden sword of Hercules. Cancer Res. 2011;71(21):6576–82.
49. Clem BF, Clem AL, Yalcin A, Goswami U, Arumugam S, Telang S, Trent JO, Chesney J. A novel small molecule antagonist of choline kinase-alpha that simultaneously suppresses MAPK and PI3K/AKT signaling. Oncogene. 2011;30(30):3370–80.
50. Vander Heiden MG, Christofk HR, Schuman E, Subtelny AO, Sharfi H, Harlow EE, Xian J, Cantley LC. Identification of small molecule inhibitors of pyruvate kinase M2. Biochem Pharmacol. 2010;79(8):1118–24.
51. Wolf A, Agnihotri S, Micallef J, Mukherjee J, Sabha N, Cairns R, Hawkins C, Guha A. Hexokinase 2 is a key mediator of aerobic glycolysis and promotes tumor growth in human glioblastoma multiforme. J Exp Med. 2011;208(2):313–26.
52. Yalcin A, Telang S, Clem B, Chesney J. Regulation of glucose metabolism by 6-phosphofructo-2-kinase/fructose-2,6-bisphosphatases in cancer. Exp Mol Pathol. 2009;86(3):174–79.
53. Dang L, White DW, Gross S, Bennett BD, Bittinger MA, Driggers EM, Fantin VR, Jang HG, Jin S, Keenan MC, Marks KM, Prins RM, Ward PS, Yen KE, Liau LM, Rabinowitz JD, Cantley LC, Thompson CB, Vander Heiden MG, Su SM. Cancer-associated IDH1 mutations produce 2-hydroxyglutarate. Nature. 2009;462(7274):739–44.
54. Locasale JW, Grassian AR, Melman T, Lyssiotis CA, Mattaini KR, Bass AJ, Heffron G, Metallo CM, Muranen T, Sharfi H, Sasaki AT, Anastasiou D, Mullarky E, Vokes NI, Sasaki M, Beroukhim R, Stephanopoulos G, Ligon AH, Meyerson M, Richardson AL, Chin L, Wagner G, Asara JM, Brugge JS, Cantley LC, Vander Heiden MG. Phosphoglycerate dehydrogenase diverts glycolytic flux and contributes to oncogenesis. Nat Genet. 2011;43(9):869–74.
55. Sreekumar A, Poisson LM, Rajendiran TM, Khan AP, Cao Q, Yu J, Laxman B, Mehra R, Lonigro RJ, Li Y, Nyati MK, Ahsan A, Kalyana-Sundaram S, Han B, Cao X, Byun J, Omenn GS, Ghosh D, Pennathur S, Alexander DC, Berger A, Shuster JR, Wei JT, Varambally S, Beecher C, Chinnaiyan AM. Metabolomic profiles delineate potential role for sarcosine in prostate cancer progression. Nature. 2009;457(7231):910–14.
56. Possemato R, Marks KM, Shaul YD, Pacold ME, Kim D, Birsoy K, Sethumadhavan S, Woo HK, Jang HG, Jha AK, Chen WW, Barrett FG, Stransky N,

Tsun ZY, Cowley GS, Barretina J, Kalaany NY, Hsu PP, Ottina K, Chan AM, Yuan B, Garraway LA, Root DE, Mino-Kenudson M, Brachtel EF, Driggers EM, Sabatini DM. Functional genomics reveal that the serine synthesis pathway is essential in breast cancer. Nature. 2011;476(7360):346–50.
57. Zhang J, Yang PL, Gray NS. Targeting cancer with small molecule kinase inhibitors. Nat Rev Cancer. 2009;9(1):28–39.
58. Dar AC, Shokat KM. The evolution of protein kinase inhibitors from antagonists to agonists of cellular signaling. Annu Rev Biochem. 2011;80:769–95.
59. Li J, Zhu Z. Research and development of next generation of antibody-based therapeutics. Acta Pharmacol Sin. 2010;31(9):1198–207.
60. Lofblom J, Frejd FY, Stahl S. Non-immunoglobulin based protein scaffolds. Curr Opin Biotechnol. 2011; 22(6):843–48.
61. Johnson LN. Protein kinase inhibitors: contributions from structure to clinical compounds. Q Rev Biophys. 2009;42(1):1–40.
62. Chan WW, Wise SC, Kaufman MD, Ahn YM, Ensinger CL, Haack T, Hood MM, Jones J, Lord JW, Lu WP, Miller D, Patt WC, Smith BD, Petillo PA, Rutkoski TJ, Telikepalli H, Vogeti L, Yao T, Chun L, Clark R, Evangelista P, Gavrilescu LC, Lazarides K, Zaleskas VM, Stewart LJ, Van Etten RA, Flynn DL. Conformational control inhibition of the BCR-ABL1 tyrosine kinase, including the gatekeeper T315I mutant, by the switch-control inhibitor DCC-2036. Cancer Cell. 2011;19(4):556–68.
63. Zhang J, Adrian FJ, Jahnke W, Cowan-Jacob SW, Li AG, Iacob RE, Sim T, Powers J, Dierks C, Sun F, Guo GR, Ding Q, Okram B, Choi Y, Wojciechowski A, Deng X, Liu G, Fendrich G, Strauss A, Vajpai N, Grzesiek S, Tuntland T, Liu Y, Bursulaya B, Azam M, Manley PW, Engen JR, Daley GQ, Warmuth M, Gray NS. Targeting Bcr-Abl by combining allosteric with ATP-binding-site inhibitors. Nature. 2010;463(7280):501–6.
64. Davis MI, Hunt JP, Herrgard S, Ciceri P, Wodicka LM, Pallares G, Hocker M, Treiber DK, Zarrinkar PP. Comprehensive analysis of kinase inhibitor selectivity. Nat Biotechnol. 2011;29(11):1046–51.
65. Eglen R, Reisine T. Drug discovery and the human kinome: recent trends. Pharmacol Ther. 2011;130(2):144–56.
66. Zhou W, Ercan D, Chen L, Yun CH, Li D, Capelletti M, Cortot AB, Chirieac L, Iacob RE, Padera R, Engen JR, Wong KK, Eck MJ, Gray NS, Janne PA. Novel mutant-selective EGFR kinase inhibitors against EGFR T790M. Nature. 2009;462(7276):1070–74.
67. Zhang C, Habets G, Bollag G. Interrogating the kinome. Nat Biotechnol. 2011;29(11):981–83.
68. Anastassiadis T, Deacon SW, Devarajan K, Ma H, Peterson JR. Comprehensive assay of kinase catalytic activity reveals features of kinase inhibitor selectivity. Nat Biotechnol. 2011;29(11):1039–45.
69. Stommel JM, Kimmelman AC, Ying H, Nabioullin R, Ponugoti AH, Wiedemeyer R, Stegh AH, Bradner JE, Ligon KL, Brennan C, Chin L, DePinho RA. Coactivation of receptor tyrosine kinases affects the response of tumor cells to targeted therapies. Science. 2007;318(5848):287–90.
70. Gossage L, Eisen T. Targeting multiple kinase pathways: a change in paradigm. Clin Cancer Res. 2010;16(7):1973–78.
71. Knight ZA, Lin H, Shokat KM. Targeting the cancer kinome through polypharmacology. Nat Rev Cancer. 2010;10(2):130–37.
72. Bostrom J, Yu SF, Kan D, Appleton BA, Lee CV, Billeci K, Man W, Peale F, Ross S, Wiesmann C, Fuh G. Variants of the antibody herceptin that interact with HER2 and VEGF at the antigen binding site. Science. 2009;323(5921):1610–14.
73. Robinson MK, Hodge KM, Horak E, Sundberg AL, Russeva M, Shaller CC, von Mehren M, Shchaveleva I, Simmons HH, Marks JD, Adams GP. Targeting ErbB2 and ErbB3 with a bispecific single-chain Fv enhances targeting selectivity and induces a therapeutic effect in vitro. Br J Cancer. 2008;99(9):1415–25.
74. Metz JT, Johnson EF, Soni NB, Merta PJ, Kifle L, Hajduk PJ. Navigating the kinome. Nat Chem Biol. 2011;7(4):200–2.
75. Arteaga CL, Baselga J. Tyrosine kinase inhibitors: why does the current process of clinical development not apply to them? Cancer Cell. 2004;5(6):525–31.
76. Andersen JN, Sathyanarayanan S, Di Bacco A, Chi A, Zhang T, Chen AH, Dolinski B, Kraus M, Roberts B, Arthur W, Klinghoffer RA, Gargano D, Li L, Feldman I, Lynch B, Rush J, Hendrickson RC, Blume-Jensen P, Paweletz CP. Pathway-based identification of biomarkers for targeted therapeutics: personalized oncology with PI3K pathway inhibitors. Sci Transl Med. 2010;2(43):43ra55.
77. 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(7287):427–30.
78. Hatzivassiliou G, Song K, Yen I, Brandhuber BJ, Anderson DJ, Alvarado R, Ludlam MJ, Stokoe D, Gloor SL, Vigers G, Morales T, Aliagas I, Liu B, Sideris S, Hoeflich KP, Jaiswal BS, Seshagiri S, Koeppen H, Belvin M, Friedman LS, Malek S. RAF inhibitors prime wild-type RAF to activate the MAPK pathway and enhance growth. Nature. 2010;464(7287):431–35.
79. LoRusso PM, Anderson AB, Boerner SA, Averbuch SD. Making the investigational oncology pipeline more efficient and effective: are we headed in the right direction? Clin Cancer Res. 2010;16(24): 5956–62.
80. Tursz T, Andre F, Lazar V, Lacroix L, Soria JC. Implications of personalized medicine—perspective from a cancer center. Nat Rev Clin Oncol. 2011;8(3):177–83.
81. Kim ES, Herbst RS, Wistuba II, Lee JJ, Blumenschein GR, Tsao A, Stewart DJ, Hicks ME, Erasmus J, Gupta S, Alden CM, Liu S, Tang X, Khuri FR, Tran HT, Johnson BE, Heymach JV, Mao L, Fossella F, Kies MS, Papadimitrakopoulou V, Davis SE, Lippman SM, Hong WK. The BATTLE trial: personalizing therapy for lung cancer. Cancer Discovery. 2011;1:44–53.
82. Biankin AV, Hudson TJ. Somatic variation and cancer: therapies lost in the mix. Hum Genet. 2011;130(1):79–91.

begell house, inc.
Journal Production
50 Cross Highway
Redding, CT 06896
Phone: 1-203-938-1300
Fax: 1-203-938-1304
Begell House Production Contact : journals@begellhouse.com

Dear Corresponding Author,

Effective April 2011 Begell House will no longer provide corresponding authors with a print copy of the issue in which their article appears. Corresponding authors will now receive a pdf file of the final version of their article that has been accepted for publication.

Please note that the pdf file provided is for your own personal use and is not to be posted on any websites or distributed in any manner (electronic or print). Please follow all guidelines provided in the copyright agreement that was signed and included with your original manuscript files.

Any questions or concerns pertaining to this matter should be addressed to journals@begellhouse.com

Thank you for your contribution to our journal and we look forward to working with you again in the future.

.

Sincerely,

Michelle Amoroso

Michelle Amoroso
Production Department