Molecular Pathways: Targeting the PI3K Pathway in Cancer—BET Inhibitors to the Rescue

Elias E. Stratikopoulos and Ramon E. Parsons

Abstract

The PI3K signaling pathway is a complex and tightly regulated network that is critical for many physiologic processes, such as cell growth, proliferation, metabolism, and survival. Aberrant activation of this pathway can occur through mutation of almost any of its major nodes and has been implicated in a number of human diseases, including cancer. The high frequency of mutations in this pathway in multiple types of cancer has led to the development of small-molecule inhibitors of PI3K, several of which are currently in clinical trials. However, several feedback mechanisms either within the PI3K pathway or in compensatory pathways can render tumor cells resistant to therapy. Recently, targeting proteins of the bromodomain and extraterminal (BET) family of epigenetic readers of histone acetylation has been shown to effectively block adaptive signaling response of cancer cells to inhibitors of the PI3K pathway, which at least in some cases can restore sensitivity. BET inhibitors also enforce blockade of the MAPK, JAK/STAT, and ER pathways, suggesting they may be a rational combinatorial partner for divergent oncogenic signals that are subject to homeostatic regulation. Here, we review the PI3K pathway as a target for cancer therapy and discuss the potential use of BET inhibition to enhance the clinical efficacy of PI3K inhibitors.

Clin Cancer Res; 22(11); 2605–10. ©2016 AACR.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Editor’s Disclosures

The following editor(s) reported relevant financial relationships: P.S. Steeg, reports receiving commercial research grants from Genentech.

CME Staff Planners’ Disclosures

The members of the planning committee have no real or apparent conflicts of interest to disclose.

Learning Objectives

Upon completion of this activity, the participant should have a better understanding of how the PI3K pathway can be deregulated in cancer, the resistance mechanisms that help cancer cells evade the effects of targeted therapy, and the rationale for combining kinase and epigenetic inhibitors to improve therapy.

Acknowledgment of Financial or Other Support

This activity does not receive commercial support.

Background

PI3Ks are a family of lipid kinases that phosphorylate the 3-hydroxyl group on phosphoinositides, generating second messengers that regulate several downstream pathways that are central in both normal physiology and disease (1, 2). In mammals, there are three classes of PI3Ks that differ in structure and substrate specificity, but to date, mainly class IA PI3Ks have been implicated in the etiology of various diseases, including cancer (3). Class IA PI3Ks are heterodimers composed of a p110 catalytic subunit (α, β, and δ) and a p85 regulatory subunit (encoded by three different genes that are subject to alternative splicing) that can be activated downstream of receptor tyrosine kinases (RTK), G protein–coupled receptors (GPCR), and small GTPases (4). Although PI3K was first linked to cancer almost 30 years ago when it was associated with the transforming activity of viral oncogenes (5), it was not until the early 2000s that PI3Ks were brought to the forefront of cancer research when PIK3R1 (6) and PIK3CA (7), the genes encoding p85α and p110α, respectively, were found to be frequently mutated in several types of solid tumors. Since then, multiple studies have established that PIK3CA is one of the most, if not the most, frequently mutated oncogenes in human...
cancer. Mutations are mainly clustered in two hotspots of the enzyme and can increase p110α activity through a variety of mechanisms (8–10).

In quiescent cells, p85 binds to p110, stabilizing it and inactivating its kinase activity (Fig. 1). Following growth factor stimulation, the PI3K complex is activated after binding to phosphotyrosines on receptors and adaptor proteins. The primary consequence of PI3K activation is the conversion of phosphatidylinositol-4,5-bisphosphate (PIP₂) into the short-lived second messenger phosphatidylinositol-3,4,5-trisphosphate (PIP3) on the inner leaflet of the plasma membrane. PIP3 recruits proteins to the membrane that contain a pleckstrin-homology domain, including AKT, PDK1, and mTORC2. At the membrane, PDK1 phosphorylates AKT at T308 (11) and mTORC2 phosphorylates it at S473 (12), which results in full activation of the enzyme. AKT acts as a major mediator of PI3K signaling by phosphorylating a wide range of substrates that regulate cell-cycle entry, survival, protein synthesis, RNA translation, glucose metabolism, and migration. PI3K activity is tightly controlled in cells and can be attenuated by lipid phosphatases, such as PTEN (13), INPP4B (14), and SHIP2 (15) that dephosphorylate phospholipids in positions 3, 4, and 5, respectively, on the inositol ring. PIP3 is also an important signal upstream of several pro-oncogenic signals, including SGK3 (16, 17) and PREX1/2 (18, 19).

Overactivation of the PI3K signal is one of the most frequent events in human cancers and can be achieved through alterations in most of the major nodes of the pathway (4). Activating mutations and/or amplification of the genes encoding the PI3K catalytic subunits p110α (7), and less frequently p110β (20), mutations in the p85α regulatory subunit that abrogate its p110-inhibitory activity (6, 21) as well as loss of function of the lipid phosphatases PTEN (22, 23) and INPP4B (14) can all promote PI3K activity and cellular transformation. Mutations in genes acting both upstream of PI3Ks, such as RTKs, such as EGFR and HER2 and the PI3K activator K-RAS as well as the downstream PI3K effectors AKT1-3, PDK1, TSC1/2, and mTOR, are variably present in human tumors and should also result in aberrant activation of the PI3K pathway.
Clinical–Translational Advances

Given the high frequency of PI3K pathway activation in human cancers, a significant amount of effort has been put into the development of drugs targeting several kinases throughout the pathway, especially PI3Ks. Several inhibitors targeting all PI3K isoforms (pan-PI3K) are currently in clinical trials, but although they have turned out to be reasonably well tolerated, they have shown limited efficacy as single agents (24). Partial inhibition of the signal due to limitations in the dosing, compensatory feedback mechanisms, as well as off-target effects could all account for the limited clinical responses that were observed in patients treated with pan-PI3K inhibitors. Recent preclinical studies have highlighted the divergent roles of class I PI3K catalytic isoforms depending on the genetic context. For example, HER2/Neu- and KRAS-driven tumors have been shown to rely mainly on p110α (25, 26), whereas p110β has been shown to be important in certain PI3K-deficient tumors (27–29). In light of these findings, PI3K isoform–selective inhibitors have been developed and are being tested in clinical trials with the hope to maximize target-inhibitory doses while sparing the adverse toxic effects of pan-PI3K inhibitors. In 2014, idelalisib (CAL-101) became the first p110 isoform–specific inhibitor (against p110β) that has been approved by the FDA for the treatment of chronic lymphocytic leukemias (30) and B-cell lymphomas (31). Alpelisib (BYL719) is a p110α-specific PI3K inhibitor that has shown some encouraging results in early-phase clinical trials. As a single agent, it resulted in some tumor regressions and prolonged disease control in heavily pretreated patients with various tumor types carrying a PIK3CA mutation (32). Preliminary results from the combination of BYL719 and fulvestrant, an estrogen receptor antagonist, also indicate an encouraging clinical effect. The combination of BYL719 and fulvestrant, an estrogen receptor antagonist, also indicate an encouraging clinical effect. In an ER+/PIK3CA–mutant breast cancer patient treated with the p110α–specific inhibitor BYL719, loss of PTEN expression (which could likely switch dependence on the p110β isoform) was present in multiple metastatic lesions (44). Markedly, treatment of xenografts derived from the BYL719-resistant lesions with the p110β–selective inhibitor AZD6482 was able to restore sensitivity. Concurrent inhibition of multiple PI3K isoforms or the use of dual-isoform PI3K inhibitors might be necessary for the treatment of tumors that have become refractory to isoform-specific inhibitors. Compounds that would be active against only the mutant PIK3CA should minimize increased systemic insulin production that is seen in the clinic upon treatment with p110α inhibitors, and these agents are highly anticipated in the field. Finally, activation of several compensatory pathways has been documented to drive resistance to PI3K inhibitors and could therefore inform combinatorial treatments. Clinical trials are currently evaluating preclinical findings of synergy between PI3K inhibition and antineoplastic therapy (45), as well as inhibitors against PARP (46), cyclin-dependent kinases 4 and 6 (CDK4/6; ref. 47), and MEK (37, 48).

Within the past year, inhibition of BET proteins has emerged as a potential therapeutic approach to restore sensitivity to kinase inhibitors of the PI3K pathway. The BET family of proteins consists of 4 members, BRD2–4, which are ubiquitously expressed, and BRDT, which is only expressed in germ cells. They contain two tandem bromodomains (BRD) located at the N-terminus that recognize and bind acetylated lysine residues in nucleosomal histones to facilitate the recruitment of transcription factors and chromatin organizers required in transcription initiation and elongation. Initially, the concept of targeting a bromodomain was thought to be challenging because it would involve inhibiting a protein–peptide interaction. Moreover, there was a lot of skepticism around the concept of epigenetic therapy, as inhibition of BET proteins, which are global regulators of gene transcription, was expected to have an impact on the transcriptional activity across all active genes and to be highly toxic. In 2010, two selective and potent BET protein inhibitors, JQ1 (49) and I-BET762 (50), were reported to have activity in a NUT midline carcinoma and an inflammatory disease model, respectively. Mechanistically, BET inhibition was shown to result in significant reduction of the transcript levels of only a small number of key genes in a cell– and context-specific manner (51, 52). This recent discovery of small molecules capable of blocking their lysine-binding pocket is the first successful example of pharmaceutical inhibition of epigenetic readers and has sparked intense efforts to develop novel BRD antagonists. Several compounds are currently being tested in early-phase clinical trials (Table 1) for solid tumors and hematologic malignancies, in which, for example, deregulated c-MYC, a major target of BET proteins in this context, is an important driver of tumorigenesis.
Most of these compounds share a similar thienodiazepine scaffold, whereas for some of them the exact chemical structure has not yet been disclosed (53). Notably, multiple studies have recently documented that BET inhibition can effectively block adaptive responses to inhibitors of the PI3K pathway that help cancer cells to evade the effects of the drug and develop resistance. For example, our group has shown that BET inhibitors can block BRD4 from binding to regulatory regions of genes encoding several RTKs that are frequently induced by treatment with pictilisib (GDC-0941), a pan-PI3K inhibitor (Fig. 1). As a result, combined PI3K and BET inhibition can prolong blockade of the PI3K signal and induce cell death in multiple tumor cell lines (54). BET proteins have also been shown to regulate sensitivity of breast cancer cells to treatment with lapatinib, an EGFR/HER2 inhibitor, by blocking the expression/phosphorylation of kinases (55) and/or activation of the FOXO/c-MYC axis (56). Finally, inhibitors of BET proteins have shown synergism with mTOR inhibitors in multiple tumor models (57–59), suggesting that blockade of the epigenetic regulation of the PI3K pathway may present an opportunity to overcome resistance to kinase inhibitor therapy.

Several groups have investigated BET inhibition as a potential therapeutic approach to potentiate the effect of targeted therapy against oncogenic drivers in other signaling pathways. For example, combinations of JQ1 and TK inhibitors, such as imatinib and JAK inhibitor I, were shown to synergize and induce apoptosis in leukemias and lymphomas driven by constitutive STAT5 activation (60). Using an anticancer drug library containing 180 small-molecule inhibitors, Jing and colleagues identified that combined BET inhibition and MEK inhibition suppressed both cell proliferation and survival in an ovarian cancer model (61). In an ER+/tamoxifen–resistant breast cancer model, JQ1 induced a strong growth-inhibitory effect when it was combined with fulvestrant (62). These studies highlight the ability of BET inhibition to synergize with inhibition of diverse oncogenic signals, which could be due to their unique ability to interfere with the adaptive feedback expression of a few relevant genes to restore signaling homeostasis depending on the genetic context (Fig. 1). However, whether there will be a therapeutic window for any of the above combinatorial therapies in the clinic remains to be seen.

A striking development in BRD inhibition research is the recent finding that BET proteins are unanticipated targets of certain widely used kinase inhibitors. The first such report was by Martin and colleagues, who discovered that the potent CDK inhibitor dinaciclib interacts with the acetyl–lysine recognition site of the bromodomain testis-specific protein BRDT (63). Soon after that, two new studies (64, 65) identified more than a dozen kinase inhibitors that possess cross-reactivity toward BRD4 with nanomolar potencies, including the JAK2 inhibitor TG101209 and the PLK1 inhibitor BI2536, which is now being evaluated in phase I/II clinical trials for acute myeloid leukemia and non–small cell lung cancer. This finding has triggered the development of more potent dual BET/PLK1 inhibitors using a structure–activity relationship study (66). Notably, LY94002, which has been routinely used to block PI3K activity, was also recently shown to interact with BRD2-4 (67). The above studies provide the framework for the rational design of next-generation dual BET/kinase inhibitors. The simultaneous inhibition of two structurally and functionally unrelated proteins by a single drug may provide a new opportunity when the application of combination therapies poses significant clinical challenges.

Conclusions

The concept of oncogene addiction described by the dependence of certain tumors for their growth and survival on a limited number of mutational events has fueled the development of targeted therapies. The PI3K signaling pathway has attracted a great deal of interest due to its involvement in a large fraction of human tumors, and several inhibitors targeting oncogenic kinases throughout the pathway are currently being tested in clinical trials. However, preclinical studies and past experience with other kinase-based therapies suggest that escape mechanisms and drug resistance may eventually limit the efficacy of these compounds as monotherapies. Concurrent inhibition of PI3K and BET proteins may provide an alternative for durable inhibition of the oncogenic signal, and clinical trials could test the safety and efficacy of such combinations.

Authors’ Contributions

Conception and design: E.E. Stratikopoulos, R.E. Parsons

Analysis and interpretation of data [e.g., statistical analysis, biostatistics, computational analysis]: R.E. Parsons

Writing, review, and/or revision of the manuscript: E.E. Stratikopoulos, R.E. Parsons

Grant Support

R.E. Parsons is supported by the NCI of the NIH under award number R01CA129432 and a Stand Up To Cancer Dream Team Translational Research Grant (SU2C-AACR-DT0209). Stand Up To Cancer is a program of the Entertainment Industry Foundation. Research grants are administered by the American Association for Cancer Research, the scientific partner of SU2C.

Received February 9, 2016; accepted March 16, 2016; published online June 1, 2016.
References


Molecular Pathways: Targeting the PI3K Pathway in Cancer—BET Inhibitors to the Rescue

Elias E. Stratikopoulos and Ramon E. Parsons


Updated version
Access the most recent version of this article at:
http://clincancerres.aacrjournals.org/content/22/11/2605

Cited articles
This article cites 64 articles, 21 of which you can access for free at:
http://clincancerres.aacrjournals.org/content/22/11/2605.full#ref-list-1

E-mail alerts
Sign up to receive free email-alerts related to this article or journal.

Reprints and Subscriptions
To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions
To request permission to re-use all or part of this article, contact the AACR Publications Department at permissions@aacr.org.