Navigating the Therapeutic Complexity of PI3K Pathway Inhibition in Melanoma

Lawrence N. Kwong and Michael A. Davies

Abstract
Melanoma is entering into an era of combinatorial approaches to build upon recent clinical breakthroughs achieved by novel single-agent therapies. One of the leading targets to emerge from the growing understanding of the molecular pathogenesis, heterogeneity, and resistance mechanisms of melanomas is the phosphoinositol 3-kinase (PI3K)–AKT pathway. Multiple genetic and epigenetic aberrations that activate this pathway have been identified in melanomas de novo and in acquired resistance models. These developments have been paralleled by the establishment of models for preclinical testing and the availability of compounds that target various effectors in the pathway. Thus, in addition to having a strong rationale for targeting, the PI3K–AKT pathway presents an immediate clinical opportunity. However, the development of effective strategies against this pathway must overcome several key challenges, including optimizing patient selection, overcoming feedback loops, and pathway cross-talk that can mediate resistance. This review discusses the current understanding and ongoing research about the PI3K–AKT pathway in melanoma and emerging strategies to achieve clinical benefit in patients by targeting it. Clin Cancer Res; 19(19); 5310–9. ©2013 AACR.

Introduction
The phosphoinositol 3-kinase (PI3K)–AKT cascade is one of the most studied pathways in cancer. The pathway is a critical regulator of many essential physiologic processes that are critical to the aggressive nature and behavior of malignant cells. Previous studies have shown that the pathway is among the most frequent targets of genetic aberrations across many types of cancer (1). These alterations include mutations and copy number changes within the core components of the pathway, as well as alterations in genes that use that pathway as a critical effector [i.e., receptor tyrosine kinases (RTK)]. For all of these reasons, the PI3K–AKT pathway has also been the focus of aggressive pharmacologic development and testing (2, 3).

The high prevalence of activating mutations in BRAF and NRAS in cutaneous melanomas supports a critical role for activation of the RAS–RAF–MEK–ERK pathway in the pathogenesis of this disease (4). However, multiple lines of evidence have also shown a significant role for the PI3K–AKT pathway. This review highlights some of the key findings about the PI3K–AKT pathway in melanoma, and the rationale, approaches, and challenges to the development of effective therapeutic approaches against it.

Activation of the PI3K–AKT Pathway in Melanoma
The physiologic regulation of the PI3K–AKT cascade is shown in Fig. 1 (5). PI3K, which consists of a dimer of catalytic (i.e., p110) and regulatory (i.e., p85) subunits, can be activated by multiple signals, including RTKs, RAS proteins, and cell–cell contacts, among others. Activated PI3K phosphorylates phosphatidylinositol in the plasma membrane at the 3’-OH group. These 3’-phosphoinositides attract proteins that contain a pleckstrin homology domain to the cell membrane, including the serine-threonine kinase AKT. AKT, which has three isoforms (AKT1/2/3), is phosphorylated at two critical and conserved residues, Thr308 (by PDK1) and Ser473 (by the mTORC2 complex), which fully activate its catalytic activity. Activated AKT then phosphorylates a number of effector proteins, thereby regulating multiple key cellular processes, including proliferation, survival, motility, metabolism, angiogenesis, and more. PTEN regulates the activity of the pathway by dephosphorylating phosphatidylinositol at the 3’-position, thereby antagonizing the activity of PI3K (6). Multiple other lipid and protein phosphatases also regulate various steps and effectors in the pathway (7).

The PI3K–AKT pathway is activated multiple ways in melanoma. The two most common and studied events are activating mutations in the oncogene NRAS (15–20%) and loss of expression and/or function of the tumor suppressor PTEN (20–30%; ref. 4). Similar to BRAF and NRAS mutations in the RAS–RAF–MEK–ERK signaling pathway, NRAS mutations and PTEN mutations/deletions are largely mutually exclusive. In contrast, PTEN loss commonly occurs in melanomas with activating BRAF mutations, resulting in concurrent activation of the RAS–RAF–MEK–ERK and PI3K–AKT pathways (8–10). The general mutual exclusivity...
of NRAS mutations and PTEN loss in melanoma is thought by many to be attributable to the fact that both events activate the PI3K–AKT pathway, thus rendering the presence of both alterations in the same tumor functionally redundant. However, similar to findings in other tumor types, quantitative analysis of melanoma cell lines and clinical specimens has shown that melanomas with PTEN loss consistently have higher levels of AKT activation than those with NRAS mutations (11–13). Furthermore, experiments in a RAS-mutant melanoma genetically engineered mouse model (GEMM) showed that loss of PTEN increased invasiveness and metastatic potential (14). Although rare, deletions and mutations of PTEN have been detected in some melanomas with activating NRAS mutations, including in two recent whole exome sequencing studies of >100 melanomas, which also detected PTEN alterations in melanomas with wild-type BRAF and NRAS (15, 16). However, these data should be interpreted with caution, as no standardized protocol has been established for defining PTEN deletions. Preliminary analysis of The Cancer Genome Atlas (TCGA) data suggests that such a standard should take into account both copy number and focality, and would decrease the discrepancies between studies. Additional studies support that PTEN expression can be regulated epigenetically, including by microRNAs and the PTENP1 pseudogene (17–20). A more complete understanding of the prevalence, pattern, molecular causes, and clinical associations of PTEN loss will likely be possible with the completion of the ongoing melanoma TCGA effort, which will include DNA-, RNA-, and protein-based analyses of up to 500 clinically annotated melanoma specimens.

The functional significance of PTEN loss has been studied extensively in the setting of melanomas with activating BRAF mutations. To date, nearly all published patient-derived melanoma cell lines with complete loss of PTEN have concurrent BRAF mutations (11, 21–24). This strong association with BRAF mutations has also been shown functionally in GEMM. Although expression of the BRAF
BRAF (42). Although the low prevalence of these subtypes, and their general mutual exclusivity with c-Kit mutations, suggested that signaling by mutant KIT proteins might activate the RAS–RAF–MEK–ERK pathway, functional studies in cell lines have shown activation of, and in some studies dependence upon, the PI3K–AKT pathway (43–45). One study has also reported frequent (~20%) somatic mutations in the ERBB4 gene (46). Although these mutations do not cluster in any functional domain, preclinical studies suggested that multiple mutant forms of the encoded ERBB4 protein activated the PI3K–AKT pathway. However, recent whole exome-sequencing studies did not identify ERBB4 as a significantly mutated gene by the algorithms used in those analyses (15, 16). In addition to genetic events, it seems that epigenetically mediated activation of RTKs plays a role in melanoma, specifically in resistance to BRAF inhibitors. Two different groups identified increased expression and activation of different RTKs [platelet-derived growth factor β (PDGFb) and insulin-like growth factor receptor (IGF1R), respectively] in progressively tumors and melanoma cell lines with acquired resistance to BRAF inhibitors (47–49). Both groups showed that the activation of these RTKs did not rescue the activity of the mitogen-activated protein kinase (MAPK) pathway but instead caused compensatory activation of the PI3K–AKT pathway. Importantly, no mutations or amplification in the genes encoding the RTKs were detected in the cell lines. Similar compensatory activation of this pathway via IGF1R was also reported in human melanoma cells with de novo resistance to killing by MEK inhibitors (29). More recently, two different groups showed that secretion of hepatocyte growth factor by nontransformed cells in the tumor microenvironment results in PI3K–AKT pathway activation in melanoma cells (50, 51). This interaction caused resistance to BRAF inhibitors in vitro and was correlated with inferior clinical outcomes in patients.

### PI3K–AKT Pathway Inhibitors

The multiple ways in which the PI3K–AKT pathway is activated in melanoma, and existing evidence for a functional role in progression and resistance, support the rationale to target it therapeutically. Indeed, similar evidence in multiple tumor types has led to the development of multiple classes of inhibitors against this pathway. Classes of agents include inhibitors of PI3K (pan-isofom and isoform-specific), dual PI3K–mTOR, AKT, and mTOR (mTORC1 and dual mTORC1/2 inhibitors; Table 1). Multiple agents are available in each class, many of which are currently undergoing clinical evaluation in patients. Although the availability of this spectrum of agents presents a tremendous opportunity, a key challenge for a relatively rare disease such as metastatic melanoma is to rationally use and prioritize these agents to determine their clinical value effectively and efficiently.

Experimental evidence from other tumor types supports that different ways of activating the PI3K–AKT pathway result in functional dependence upon different effectors, and thus sensitivity to different classes of therapeutic agents.
Melanomas with loss of PTEN represent a high-priority opportunity, due to the high prevalence of this alteration de novo, the availability of models for functional testing, and the evidence for a role in resistance to MAPK pathway inhibitors. Previous studies in multiple tumor types showed that loss of PTEN correlates with marked dependence on AKT and sensitivity to AKT inhibition in gene knockdown experiments (13). However, recently reported experiments using the BRAF-mutant, PTEN-null melanoma GEMM suggest superior in vivo tumor growth inhibition with PI3K inhibitors than with AKT inhibitors (52, 53). Although these results are interesting, it remains unclear how well this model will reflect results in BRAF-mutant, PTEN-null melanomas in patients, which will likely have significant

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Table 1. PI3K pathway inhibitors currently in clinical trials for any cancer
heterogeneity and additional molecular alterations that cannot be modeled easily in GEMM systems. Testing of dual PI3K–mTOR inhibitors in melanoma cell lines has shown that these agents are broadly inhibitory, and often superior to the inhibition achieved by PI3K or mTOR inhibition alone (54, 55). Although improved antitumor activity is preferred, a key question is whether this will translate into an acceptable therapeutic index in patients due to the broad physiologic functions of PI3K and mTOR. Recently reported experiments in other models have suggested that the efficacy of AKT inhibitors is relatively selective for tumors with PTEN loss (56). It is possible that this selectivity for cells with loss of PTEN will translate into selective killing of tumor cells in patients with AKT inhibitors at clinically tolerated doses, even if they are less potent. Rapamycin and its analogues, which inhibit mTORC1, are reasonably well tolerated clinically, as shown by long-standing use in patients who have undergone organ transplantation. However, mTORC1 inhibitors have not shown significant clinical activity as single agents in metastatic melanoma patients, or in combination with RAF inhibitors (57–59). As discussed below, this lack of activity may be due to compensatory hyperactivation of AKT due to inhibition of an mTORC1-mediated negative feedback loop within the PI3K pathway. In contrast, dual mTORC1/2 inhibitors block this upregulation through the additional blockade of mTORC2-mediated phosphorylation/activation of AKT, and thus may represent a more effective strategy to test the effects of mTOR inhibition (29). A recent study has also shown that genetic inhibition of PDK1 can induce melanoma regression (60), supporting the rationale for testing of PDK1 inhibitors in melanoma as they are developed clinically.

One strategy to achieve significant pathway inhibition clinically with an acceptable therapeutic index is the use of isoform-specific PI3K inhibitors. Genetic studies in mouse models have shown that the PI3K catalytic subunit p110α is predominantly responsible for mediating growth factor signaling from RTKs, but it is largely dispensable for pathway activation in tumors with PTEN loss. Cells with PTEN loss instead seem to depend largely on p110β to activate the pathway, drive proliferation, and mediate tumorigenesis in vivo (61, 62). Testing of a p110β-selective inhibitor in a panel of >400 cancer cell lines showed significantly greater activity in lines with loss of PTEN than in those with PTEN intact (63). However, despite the overall trend, some PTEN-intact cell lines were sensitive, and a number of PTEN-null cell lines were resistant. Clinical testing of two different p110β-selective inhibitors (GSK2636771, SAR260301) is currently ongoing, with planned analysis of PTEN built into both studies (www.clinicaltrials.gov). Other PI3K isoform-specific inhibitors, particularly BYL719 (p110α) and CAL-101 (p110β), have been well tolerated and showed clinical efficacy in other cancer types (64). The use of a p110α-selective inhibitor may be a rational approach, in particular, for tumors with PI3K–AKT pathway activation mediated by RTKs, but to date there are no published experimental data testing this hypothesis in melanoma.

Another strategy to optimize the therapeutic index of PI3K–AKT pathway inhibitors is the use of alternative dosing schedules. Multiple studies have shown that induction of apoptosis by BRAF or MEK inhibitors in melanoma cell lines with activating BRAF mutations generally is not observed until the MAPK pathway has been suppressed for 48 to 72 hours (29, 65). In contrast, when PI3K pathway inhibitors are combined with those agents not only is apoptosis increased but it is also generally induced at much earlier timepoints (i.e., 24 hours or less; refs. 29, 55). This suggests that relatively short-term exposure to PI3K–AKT pathway inhibitors may be effective clinically. This strategy is similar to that used conventionally with chemotherapy agents, in which dosing regimens have been developed to deliver the maximally tolerated doses of agents intermittently (i.e., every 7, 14, or 21 days). The clinical development of targeted therapies instead has generally used continuous dosing regimens. As one of the concerns about the clinical development of PI3K–AKT pathway inhibitors has been whether sufficient pathway inhibition is being achieved, the use of high, intermittent dosing may overcome this hurdle. Indeed, intermittent dosing of the combination of a MEK and a PI3K inhibitor exhibited marked antitumor activity in vivo in multiple xenograft models, including melanoma (66). Although this strategy can be explored empirically in mouse models, one of the critical challenges to the rational development of this strategy is the identification of pharmacodynamic markers that correlate with the achievement of clinically effective pathway inhibition by PI3K–AKT inhibitors (67). Notably, PI3K inhibitors generally produce marked inhibition of AKT activation at doses that are much lower than those that correlate with antiproliferative and/or proapoptotic effects. The identification of targets, and/or the degree of target modulation, that will correspond to clinical benefit, similar to what has been shown for P-ERK and BRAF inhibitors (68), will facilitate the preclinical development and clinical evaluation of candidate agents and dosing regimens.

Feedback Loops and Cross-Talk

Growing experience with effective targeted therapies, particularly in melanoma with selective BRAF inhibitors, has shown that compensatory signaling within and between signaling pathways can be critical to both clinical activity and the emergence of resistance (69–71). Consistent with this experience, effective clinical targeting of the PI3K–AKT pathway will likely also need to account for and overcome complex feedback loops that blunt the activity of single-target inhibitors against it. The seminal example is the feedback induction of AKT phosphorylation by mTORC1 inhibitors, such as rapamycin and RAD001 (Fig. 2). In these studies (72, 73), the mTORC1 complexes were found to negatively regulate IRS1 at baseline, a critical second
messenger from the IGF1R to PI3K. mTORC1 inhibitor–mediated relief of this negative loop activates PI3K, AKT, and sometimes ERK (74, 75), promoting cell survival (Fig. 2B). This particular feedback has been shown in a variety of cancers (72–76) including melanoma (28, 77).

More complex feedback perturbations are generated by PI3K and AKT inhibitors (Fig. 3). In breast, lung, and prostate cancer cell lines, these inhibitors induced the FOXO-mediated transcription of multiple RTKs, most commonly HER3 and IGF1R [78–80 (Fig. 3B)]. Independent
studies showed that these RTKs are capable of transducing signals to both the PI3K–AKT (78) and the RAS–RAF–MEK–ERK pathways (80), potentially reinforcing mutual oncogenic cross-talk. Only when these RTKs were targeted by RNA interference knockdown or by small-molecule inhibitors (lapatinib, NVP-AEW541) were these feedback activations extinguished. Indeed, combinations of the PI3K and RTK inhibitors displayed synergy in xenograft models (79–81), supporting their therapeutic value. However, one point of contention is whether dual mTORC1/2 inhibitors (also referred to as mTORC catalytic inhibitors) can induce RTKs, as some studies explicitly observe this (72, 80, 81) whereas others do not (78, 79), even in the same cell type. These varied results may be due to differences in whether the readout is mRNA or protein, as posttranslational modifications and/or protein turnover rates can result in discordant levels (82, 83), or whether the inhibitor hits both the mTORC1 and mTORC2 complexes. Regardless, these overall results serve as an important caution for the future development of PI3K inhibition in melanoma and reveal potential cotargets to suppress the feedback activity (Figs. 2 and 3).

Dual PI3K–mTOR inhibitors (84) improve on these single-target agents by providing a built-in inhibition of the mTOR feedback loops. Characterization of multiple dual inhibitors in melanoma cell lines has shown a potent and durable extinction of pAKT and its downstream targets, matching or even exceeding the effects of combining single PI3K and mTOR inhibitors (54, 77). Indeed, BEZ235 has shown preliminary success in various preclinical models, particularly in combination with MEK inhibitors (77, 85, 86). Relevantly, in a mouse model of melanoma, the combination of BEZ235 with the MEK inhibitor AZD6244 produced a 37% partial response rate (>30% decrease in tumor volume; ref. 87). Various clinical trials are currently in progress with this class of drugs, although whether pharmacokinetic and toxicity issues can be optimized remains to be seen (88).

The existence of these and other feedback loops suggests that pharmacodynamic, mechanistic, and resistance tissue-based studies of PI3K–AKT pathway inhibitors in patients should optimally allow for the evaluation of multiple markers and/or pathways. Emerging proteomic technologies including phospho-RTK and reverse phase protein arrays (RPPA) facilitate such analyses by analyzing a large number of proteins in individual samples concurrently. Notably, although most experimental work to date examining markers and mechanisms of efficacy and resistance with PI3K–AKT pathway inhibitors has focused on the effects and changes observed in tumor cells, it is becoming clear that anticancer treatments also have marked effects on the host, including the immune system and the tumor microenvironment. In melanoma, the durable efficacy of immunotherapies (89) and a growing appreciation of the effects of MAPK-pathway inhibitors on the antitumor response (90–93) mandate examination of the immunologic effects of PI3K–AKT pathway inhibitors in this disease. The marked activity of p110δ-selective inhibitors in hematologic malignancies, and the long-standing use of mTORC1 inhibitors (rapamycin) as immunosuppressants in transplant patients, raises the possibility that strategies that target the PI3K–AKT pathway could actually inhibit the antitumor immune response and thus blunt long-term clinical benefit. However, an improved understanding of antitumor immunology and the differential effects of various PI3K–AKT pathway inhibitors on different immune cell populations (94), coupled with strategies (i.e., isoform-specific inhibitors) that are designed to achieve selective pathway inhibition in tumors, suggests that this challenge will not be insurmountable.

Summary

The PI3K–AKT pathway remains an attractive combinatorial target to improve clinical outcomes in patients with melanoma. As described, emerging understanding and models for this pathway are facilitating the development of rational strategies. However, critical challenges remain, including matching patients to the appropriate agents; developing appropriate markers to facilitate efficient and meaningful evaluation of doses that are achieved safely in patients; and, ultimately, identifying strategies that achieve acceptable therapeutic indices.

Disclosure of Potential Conflicts of Interest

M.A. Davies has commercial research grants from GlaxoSmithKline, AstraZeneca, Genentech, Merck, Oncothyreon, and Myriad. Also, M.A. Davies is a consultant/ advisory board member for GlaxoSmithKline, Genentech, and Novartis. No potential conflicts of interest were disclosed by the other author.

Authors’ Contributions

Conception and design: L. Kwong, M.A. Davies
Writing, review, and/or revision of the manuscript: L. Kwong, M.A. Davies

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References

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