Targeting the PI3K/Akt/mTOR pathway in castration-resistant prostate cancer

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Abstract
The phosphatidylinositol 3-kinase (PI3K)/Akt/mammalian target of rapamycin (mTOR) pathway is a key signaling pathway that has been linked to both tumorigenesis and resistance to therapy in prostate cancer and other solid tumors. Given the significance of the PI3K/Akt/mTOR pathway in integrating cell survival signals and the high prevalence of activating PI3K/Akt/mTOR pathway alterations in prostate cancer, inhibitors of this pathway have great potential for clinical benefit. Here, we review the role of the PI3K/Akt/mTOR pathway in prostate cancer and discuss the potential use of pathway inhibitors as single agents or in combination in the evolving treatment landscape of castration-resistant prostate cancer.

Key Words
- castration-resistant prostate cancer (CRPC)
- PI3K inhibitors
- mTOR
- PI3K pathway
- Akt
- androgen receptor signaling
- combination therapy

Introduction
Prostate cancer is the second most common cancer and sixth leading cause of cancer-related mortality in men, accounting for 903,500 new diagnoses and 258,400 deaths per year worldwide (Jemal et al. 2011). Although the prognosis for patients with localized or regional disease is good, for patients in the United States who develop metastatic disease, the 5-year survival rate is only 29% (Siegel et al. 2012). Currently, androgen deprivation therapy through either chemical or surgical castration is the first-line therapy for metastatic disease. Response to therapy, however, is temporary, and patients invariably progress to castration-resistant prostate cancer (CRPC; Rini & Small 2002). The TAX327 trial established docetaxel plus prednisone as the standard-of-care first-line chemotherapy for CRPC (Berthold et al. 2008), and until recently, treatment of CRPC was limited, with only docetaxel-based regimens offering a survival benefit (Petrylak et al. 2004, Berthold et al. 2008). Recently, several new therapies have emerged, including the novel taxane chemotherapeutic cabazitaxel (de Bono et al. 2010), the androgen synthesis inhibitor abiraterone acetate (de Bono et al. 2011), the novel androgen receptor (AR) inhibitor enzalutamide (Scher et al. 2012), the immunotherapeutic sipuleucel-T (Kantoff et al. 2010), and the bone microenvironment-targeted radiopharmaceutical alpharadin (Radium-223; Parker et al. 2012), leading to approval or submission for approval to regulatory agencies. While these therapies have established androgen synthesis and AR signaling, microtubule dynamics, and the bone microenvironment as targets for therapeutic intervention and have provided clinical benefit to men with metastatic CRPC, the survival rate of patients with metastatic CRPC remains poor and additional therapeutic approaches are needed.
The phosphatidylinositol 3-kinase (PI3K)/Akt/mammalian target of rapamycin (mTOR) pathway is a key oncogenic signaling pathway that has been linked to tumorigenesis and resistance to both conventional and targeted anticancer therapies in a wide variety of tumor types (Courtney et al. 2010, McCubrey et al. 2011). In prostate cancer, activation of the PI3K/Akt/mTOR pathway has been strongly implicated in prostate cancer progression (Pourmand et al. 2007, Reid et al. 2010, Taylor et al. 2010). Here, we review the role of the PI3K/Akt/mTOR pathway in prostate cancer and discuss the potential of PI3K/Akt/mTOR pathway inhibitors as single agents or in combination with other agents in the currently evolving treatment landscape of CRPC.

The PI3K/Akt/mTOR signaling pathway

The PI3K/Akt/mTOR signaling pathway has a diverse array of functions, including the regulation of cellular survival, differentiation and stem cell-like properties, growth, proliferation, metabolism, migration, and angiogenesis (Guba et al. 2002, Dubrovska et al. 2009, Liu et al. 2009, Courtney et al. 2010, Furic et al. 2010, Hsieh et al. 2012). There are three classes of PI3Ks that are differentiated by their structural characteristics and substrate specificities (Courtney et al. 2010). Class I PI3Ks are activated by receptor tyrosine kinases (RTKs), G-protein-coupled receptors, and some oncogenes, such as rat sarcoma oncogene (RAS), and can be further subdivided into class IA and IB, of which class IA PI3Ks are most frequently implicated in cancer. Class IA PI3Ks consist of two subunits: a regulatory subunit, p85, and a catalytic subunit, p110. There are three isoforms of p85 (p85α, p85β, and p55γ) encoded by the genes PIK3R1, PIK3R2, and PIK3R3 respectively and three isoforms of class IA p110α, β, and δ encoded by the genes PIK3CA, PIK3CB, and PIK3CD respectively (Courtney et al. 2010). Activation of PI3K leads to the phosphorylation of phosphatidylinositol 4,5-bisphosphate [PI(4,5)P2] to phosphatidylinositol 3,4,5-trisphosphate [PI(3,4,5)P3] and subsequent recruitment of Akt to the plasma membrane where Akt is activated (Liu et al. 2009, Courtney et al. 2010). Akt activation is mediated through phosphorylation at two residues: T308 by phosphoinositide-dependent kinase-1 and S473 by the mTOR complex 2 (TORC2). Both phosphorylation events are required for full activation of Akt (Sarbassov et al. 2005). Upon activation, Akt phosphorylates a host of other proteins including glycogen synthase kinase 3 (GSK3), FOXO transcription factors, and tuberous sclerosis complex (TSC) and thereby regulates a range of cellular processes, including protein synthesis, cell survival, proliferation, and metabolism (Fig. 1; Liu et al. 2009, Courtney et al. 2010). In prostate cancer, PI3K signaling has been shown to repress AR transcriptional activity, illustrating the lineage-specific complexity of this pathway (Fig. 2; Carver et al. 2011).

mTOR is a serine threonine kinase and is the catalytic subunit of the functionally distinct TORC1 and TORC2. TORC1 is a major downstream effector of Akt signaling and is activated via Akt-mediated inhibition of TSC. Depending on available nutrients and the cellular environment, TORC1 controls the growth of the cell through phosphorylation of S6K and the eukaryotic initiation factor 4E-binding protein 1 (4E-BP1; Sparks & Guertin 2010). TORC2 does not bind to and is generally insensitive to rapamycin and has been shown to signal independently of TORC1. TORC2 is believed to mediate cell proliferation and cell survival through phosphorylation of its substrates, which include Akt, serum-, and glucocorticoid-induced protein kinase (SGK), and protein kinase C (Sparks & Guertin 2010).

PI3K/Akt/mTOR signaling is negatively regulated by the tumor suppressor phosphatases and tensin homolog (PTEN) and inositol polyphosphate-4-phosphatase, type II (INPP4B), which convert PI(3,4,5)P3 to PI(4,5)P2 and PI(3,4)2 to PI(3)P respectively. TORC1-activated S6K has also been shown to negatively regulate PI3K/Akt/mTOR by phosphorylating TORC2, resulting in a reduction in TORC2-dependent S473 phosphorylation of Akt (Dibble et al. 2009). In addition, S6K negatively regulates RTK signaling and hence PI3K/Akt/mTOR and RAS/RAF/MEK signaling by phosphorylating and causing the degradation of insulin receptor substrate 1 (IRS-1; O’Reilly et al. 2006, Carracedo et al. 2008, Rodrik-Outmezguine et al. 2011).

The PI3K/Akt/mTOR signaling pathway in prostate cancer

In prostate cancer, alterations of components of the PI3K/Akt/mTOR pathway, including mutation, altered expression, and copy number alterations, have been reported in 42% of primary prostate tumors and 100% of metastatic tumors (Taylor et al. 2010). These alterations lead to increased PI3K/Akt/mTOR signaling activity, and the constellation of abnormalities include decreased expression of the inhibitory phosphatases PTEN, INPP4B, and PH and leucine-rich repeat protein phosphatase (PHLPP; a negative regulator of Akt); activating mutations in the PI3K catalytic gene PIK3CA; and decreased expression of the PI3K regulatory genes PIK3R1 and PIK3R3 (Fig. 3). Furthermore, androgens cause the...
TORC2 complex components, rapamycin-insensitive companion of mTOR, and stress-activated protein kinase-interacting protein 1 to accumulate in the nucleus, which stimulates TORC2 to activate Akt (Fang et al. 2012). Prostate cancer development in the setting of PTEN loss requires TORC2 activity (Guertin et al. 2009). Both PTEN loss and Akt activation have been associated with poor clinical outcome (Kreisberg et al. 2004), biochemical recurrence after radical prostatectomy (Ayala et al. 2004, Bedolla et al. 2007), and resistance to radiation (Skvortsova et al. 2008) and chemotherapy (Grunwald et al. 2002, Qian et al. 2010). In addition, PTEN loss has also been shown to predict for shorter time to metastasis (Lotan et al. 2011), and castration-resistant growth has been shown to be an intrinsic property of PTEN-null prostate cancer cells, independent of cancer developmental stage (Mulholland et al. 2011). Preclinical studies suggest that the PI3K/Akt/mTOR pathway is important in maintaining a cancer stem cell population (Dubrovsk et al. 2009) and is involved in epithelial-to-mesenchymal transition (EMT) in prostate cancer cells (Lim et al. 2011, Mulholland et al. 2012). Furthermore, the loss of PTEN results in upregulation of the transforming growth factor β/SMAD4 pathway and subsequent silencing or loss of SMAD4 may contribute to the metastatic propensity of PTEN-null prostate cancer (Ding et al. 2011). Taken together, these data suggest that the PI3K/Akt/mTOR pathway is fundamental to the metastatic potential of prostate cancer and provide a strong rationale for targeting the PI3K/Akt/mTOR pathway in this disease.

Cross talk with other signaling pathways

In addition to its direct effects, the PI3K/Akt/mTOR pathway contributes to prostate cancer development and progression through interacting with other cell signaling pathways.
pathways important for cellular survival, growth, and differentiation, including the AR and the RAS/RAF/MEK signaling pathways (Fig. 2).

AR signaling is critical for the development and function of the normal prostate gland and remains important upon neoplastic transformation, which is the basis for androgen deprivation therapy (Chen et al. 2008). Amplification of AR signaling, mutations in the ligand-binding domain of AR, and induction of AR splice variants have been shown to promote castration-resistant progression (Watson et al. 2010, Mostaghel et al. 2011). In addition, prostate cancer cells can develop the machinery necessary for autocrine androgen production that is capable of activating AR in the face of low serum testosterone levels (Montgomery et al. 2008). These data support the importance of active hormonal signaling in CRPC progression and explain the success of novel androgen synthesis inhibitors, such as abiraterone acetate, and AR antagonists, such as enzalutamide, even in the castrate-resistant state. However, responses to abiraterone acetate and enzalutamide are not universal, and when present, are often short-lived, lasting ~6–8 months in the post-docetaxel CRPC setting (de Bono et al. 2011, Scher et al. 2012).

The PI3K/Akt/mTOR and AR signaling pathways have recently been shown to regulate each other through complex reciprocal feedback mechanisms (Fig. 2; Wen et al. 2000, Lin et al. 2001, Li et al. 2008, Sarker et al. 2009, Carver et al. 2011, Mulholland et al. 2011). PI3K/Akt/mTOR signaling inhibits AR signaling via feedback mechanisms of Akt-mediated regulation of AR activity include direct phosphorylation of AR (Wen et al. 2000, Lin et al. 2001); Akt-mediated regulation of a variety of transcription factors, including FOXO3a, and NF-κB; and Akt-mediated regulation of β-catenin via GSK3β (Li et al. 2008, Sarker et al. 2009).
inhibition of human epidermal growth factor 2/3 (HER2/3) kinases, which have been shown to promote AR stability and transcriptional activity (Carver et al. 2011). In addition, PTEN increases the transcriptional activity of AR by negatively regulating the expression of the transcription factors Egr1 and c-JUN, which inhibit AR-targeted gene expression (Mulholland et al. 2011). Meanwhile, AR signaling downregulates PI3K/Akt/mTOR signaling through FK506-binding protein-5 (FKBP-5)-mediated stabilization of the Akt phosphatase PHLPP (Carver et al. 2011). Activation of the PI3K/Akt/mTOR pathway as a result of treatments targeting AR signaling or due to PTEN loss may, therefore, enable prostate cancer cells to survive and proliferate in androgen-reduced conditions. Conversely, PTEN controls the transcription of NKX3.1, which negatively regulates the AR promoter and reduces AR pathway signaling. When PTEN is lost, this brake on AR signaling is also lost (Lei et al. 2006). Therefore, inhibition of the PI3K/Akt/mTOR pathway may lead to a compensatory increase in AR activity and may promote prostate cancer progression and development of resistance to single-agent PI3K pathway inhibitor therapy. This concept of reciprocal inhibition is further supported by the recent finding that use of antiandrogens for chemoprevention actually accelerates progression to invasive prostate cancer in a PTEN-null mouse model (Jia et al. 2013). Thus, blockade of both the AR and PI3K/Akt/mTOR signaling pathways may lead to more effective anticancer activity than targeting either pathway alone.

The RAS/RAF/MEK pathway is also involved in extensive cross talk with the PI3K/Akt/mTOR pathway (Fig. 2), and activation of this pathway has been associated with decreased sensitivity to PI3K/Akt/mTOR pathway inhibitors (Ihle et al. 2009). Recently, a study of prostate cancer tissue microarrays found that the RAS/RAF/MEK pathway was significantly elevated in both primary and metastatic lesions (Mulholland et al. 2012). In murine models, PTEN deletion results in the development of prostate cancer (Wang et al. 2003); however, the combination of PTEN deletion and RAS activation significantly accelerated prostate cancer progression caused by PTEN loss, and this was accompanied by EMT and macrometastasis (Mulholland et al. 2012). Furthermore, inhibition of the RAS/RAF/MEK pathway with a MEK inhibitor (PD325901) significantly reduced metastatic progression initiated by PTEN-deficient and KRAS-activated stem/progenitor cells (Mulholland et al. 2012). Thus, cross talk
between the PI3K/Akt/mTOR pathway and the RAS/RAF/MEK pathway in prostate cancer is likely clinically important in promoting metastasis.

RTKs, which activate both the PI3K/Akt/mTOR and the RAS/RAF/MEK pathways, are in turn negatively regulated by TORC1 activity; inhibition of TORC1 leads to a relief of an inhibitory signal from S6 to IRS-1, a receptor substrate for multiple cell surface receptors, such as insulin and IGF, HER2/3 and epidermal growth factor receptor (EGFR), and others (O’Reilly et al. 2006, Carver et al. 2011, Rodrik-Outmezguine et al. 2011). Relief of this feedback inhibition with single-agent rapamycin analogs may lead to rapid compensatory PI3K and Akt re-activation and limit the pharmacodynamics and clinical impact of these agents on tumor cell survival and invasion/metastasis (Rodrik-Outmezguine et al. 2011). Combined inhibition of cell surface receptors with TORC1 inhibitors has demonstrated an ability to overcome this feedback loop and is an area of ongoing investigation. Whether combination therapy with PI3K, AR, cell surface receptor, and/or RAS/RAF/MEK pathway inhibition is needed for optimal therapy in preclinical or clinical prostate cancer is not known at this time.

**Inhibiting the PI3K/Akt/mTOR pathway in prostate cancer**

Given the importance of the PI3K/Akt/mTOR pathway in integrating cell survival signals and the high prevalence of activating PI3K/Akt/mTOR pathway alterations in prostate cancer, inhibitors of this pathway have great potential to deliver clinical benefit for men with CRPC. There are a number of agents under investigation and in the clinic today that target the PI3K/Akt/mTOR pathway for the treatment of advanced cancers. Many of these are oral agents, some are administered intravenously, and all are associated with some degree of reversible metabolic toxicity such as hyperglycemia and hyperlipidemia (Busaidy et al. 2012). Inhibition of various components of the PI3K/Akt/mTOR pathway results in differing side effect and efficacy profiles, as discussed below.

**mTOR inhibitors: early clinical experience**

The allosteric TORC1 inhibitor rapamycin and its analogs (rapalogs), including everolimus, temsirolimus, and ridaforolimus, were the first inhibitors of the PI3K/Akt/mTOR pathway to enter clinical development. In preclinical studies, these early TORC1 inhibitors were shown to revert prostatic intraepithelial neoplasia in a mouse model overexpressing human Akt (Majumder et al. 2004) and to inhibit tumor growth in mouse xenograft models derived from PTEN<sup>−/−</sup> PC-3 and PTEN<sup>+/−</sup> DU145 cells (Wu et al. 2005). Despite these promising preclinical results and the success of rapalogs in the treatment of patients with metastatic renal cell carcinoma (Motzer et al. 2008, Kwitkowski et al. 2010) and pancreatic neuroendocrine tumors (Yao et al. 2011), the clinical experience of single-agent TORC1 inhibition in men with CRPC has been disappointing, with few (if any) clinical, radiographic, or prostate-specific antigen (PSA) responses, and a relatively short time to clinical progression (2–3 months; Amato et al. 2008, George et al. 2008, Templeton et al. 2011). Moreover, in a pharmacodynamic study of rapamycin in men with intermediate- to high-risk localized prostate cancer treated before radical prostatectomy, inhibition of the TORC1 target phospho-S6 was clearly demonstrated, but no significant effects on tumor cell proliferation (Ki-67), induction of apoptosis (caspase-3 cleavage), post-treatment tumor grade or stage, or PSA levels were observed (Armstrong et al. 2010). In addition, upregulation of Akt activity was seen in some men, but again changes in Akt activity after rapamycin exposure did not correlate with any indication of treatment benefit. These data, which are supported by our observations with the TORC1 inhibitor temsirolimus in a phase II trial of men with CRPC (NCT00887640) and a single-arm study of oral rapamycin (Amato et al. 2008), establish that rapalogs are able to inhibit the intended target (TORC1) in human prostate cancer at standard dosing but do not elicit striking pathological or clinical benefits as single agents.

Several explanations likely underlie the lack of clinical activity of rapamycin analogs in advanced prostate cancer. One reason is that rapalogs do not inhibit TORC2, which activates Akt in prostate cancer cells (Sarbassov et al. 2006). This, combined with loss of S6K-mediated negative regulation of TORC2 as a result of TORC1 inhibition (Dibble et al. 2009), may therefore explain the increases in Akt activity and lack of clinical activity seen with rapalogs in prostate cancer and suggests that combined TORC1/2 inhibition may be required. In addition, paradoxical activation of cell surface receptors (such as HER2/3), AR, or RAS pathway, as discussed earlier, may explain treatment resistance. Finally, other TORC1 downstream targets, such as the eukaryotic initiation factor 4E-BP1 and the eukaryotic translation initiation factor 4E (eIF-4E) complex, are not inhibited by these agents (Furic et al. 2010). 4E-BP1 regulates translation elongation of a discrete set of key oncogenic proteins related to de-differentiation and EMT and has been directly
implicated in prostate cancer invasion and metastatic progression. Inhibition of 4E-BP1 has been reported with TORC1/2 kinase inhibitors and more upstream pathway inhibitors (Hsieh et al. 2012). Thus, while first-generation signaling inhibitors of TORC1 left this key translational oncogenic apparatus unsuppressed, second-generation TORC1/2 kinase inhibitors and further upstream PI3K pathway inhibitors may have a greater clinical impact.

**Dual TORC1/2 inhibitors**

By inhibiting both mTOR complexes, ATP-competitive dual TORC1/2 inhibitors should prevent the upregulation of Akt seen with rapalogs and lead to more complete suppression of the PI3K/Akt/mTOR pathway. Indeed, these agents have been shown to possess a greater ability to inhibit 4E-BP1 and protein synthesis and induce cell cycle arrest in several cell lines (Sparks & Guertin 2010). Furthermore, in prostate cancer, the TORC1/2 inhibitor MLN0128 was superior to TORC1 inhibition alone, as it not only prevented prostate cancer invasion and metastasis but also induced apoptosis (Hsieh et al. 2012). This improved efficacy may not simply reflect the ability of dual TORC1/2 inhibitors to inhibit both TORC1 and TORC2 but may be due to more complete inhibition of TORC1 downstream effectors, such as the eIF–4E complex. A possible caveat to improved inhibition of TORC1 is that, even in the context of TORC2 inhibition, the loss of S6K-mediated negative feedback may still activate PI3K/Akt/mTOR signaling via activation of RTKs. Supporting this view is the fact that at low concentrations (50 nM), the TORC1/2 inhibitor Torin was shown to activate Akt, and only at high concentrations (250 nM) did Torin inhibit TORC2 (Peterson et al. 2009). Despite these concerns, the TORC1/2 inhibitors MLN0128, AZD2014, DS-3078a, and OSI-027 are currently in early-stage clinical trials in solid tumors, including prostate cancer.

**Pan-PI3K inhibitors**

Pan-PI3K inhibitors target the catalytic subunits of all three isoforms of class IA PI3Ks (p110α, β, and δ) and the class IB PI3K catalytic subunit p110γ. In preclinical studies, an early pan-PI3K inhibitor, LY294002, was shown to suppress cell invasion and motility in the highly metastatic androgen-independent Dunning rat prostate cancer MLL cell line; however, this agent lacked favorable pharmacological properties and had many off-target effects (Prawettongsopon et al. 2009). Meanwhile, the PI3K inhibitors GDC-0941 and BKM120, which have improved pharmacological properties, have been shown to inhibit proliferation in the androgen-independent metastatic PC-3 cell line and halt tumor growth in xenograft mice harboring PC-3M cells (Raynaud et al. 2009, Maira et al. 2012b).

The largest clinical experience to date of a pan-PI3K inhibitor is a phase I first-in-man study of BKM120, which defined the maximum tolerated dose of BKM120 as 100 mg/day. Overall, treatment with BKM120 was well tolerated, and treatment-related adverse events included rash, hyperglycemia, diarrhea, anorexia, mood alteration (37% each), nausea (31%), and fatigue (26%). Of the 31 evaluable patients, one patient (3%) had a confirmed partial response, 16 patients (52%) had stable disease for at least 6 weeks, and seven patients (23%), including one patient with prostate cancer, remained on study for 8 months (Bendell et al. 2012). Neuropsychiatric adverse events, including reversible and generally mild-to-moderate mood alterations and depression were seen with BKM120 treatment and are thought to reflect the ability of BKM120 to cross the blood–brain barrier and inhibit the PI3K/Akt/mTOR pathway in the brain (Maira et al. 2012a, Nanni et al. 2012). In support of this, low/dysfunctional PI3K/Akt/mTOR signaling has been shown to reduce the concentration of the neurotransmitters GABA and serotonin in anxiety-related brain regions such as the amygdala and has been linked to anxiety and depression (Ackermann et al. 2008). Mood alterations observed in this phase I study of BKM120, therefore, highlight the need for close monitoring for psychiatric symptoms in patients treated with PI3K/Akt/mTOR inhibitors. Other PI3K inhibitors including GDC-0941, SAR245408, and the irreversible PI3K inhibitor PX-866 have also been shown to be well tolerated and have demonstrated signs of preliminary activity in patients with advanced solid tumors (Edelman et al. 2010, Jimeno et al. 2010, Von Hoff et al. 2010). Common adverse events included nausea, diarrhea, vomiting, fatigue, decreased appetite, dysgeusia, and rash with GDC-0941 (Von Hoff et al. 2010); skin rash with SAR245408 (Edelman et al. 2010); and nausea, vomiting, and diarrhea with PX-866 (Jimeno et al. 2010). BKM120 (as a single agent and in combination with abiraterone acetate) and PX-866 are currently under investigation in metastatic CRPC (Table 1). A limitation of single-agent PI3K pathway inhibition, as with single-agent TORC1/2 inhibition, is the possible relief of reciprocal feedback pathways that inhibit cell surface receptor, AR, and RAS pathway activation (Carver et al. 2011, Rodrik-Outmezguine et al. 2011). Thus, careful clinical pharmacodynamic studies are needed to
determine whether resistance mechanisms observed preclinically (Carver et al. 2011) are also clinically relevant. Understanding such resistance mechanisms will aid in the selection of optimal partners for combination.

Isoform-specific PI3K inhibitors

As each isoform of p110 has a distinct role (Jia et al. 2009), targeting the p110 isoform involved in a particular cancer may therefore have the advantage of an improved safety profile. PIK3CA, which encodes p110α, is mutated in 6% of primary and 16% of metastatic prostate cancers (Taylor et al. 2010), and the PIK3CA H1047R mutation has been shown to be predictive of response to pan-PI3K inhibitors (Janku et al. 2012), suggesting that p110α-specific inhibition may be effective treatment in tumors with activating PIK3CA mutations. Indeed, both the p110α isoform-specific inhibitors BYL719 (Fritsch et al. 2012) and the MLN1117 (Jessen et al. 2011) have demonstrated antitumor activity in tumor cell lines with PIK3CA mutations. However, in a PTEN-null prostate tumor model, ablation of p110β, but not p110α, impedes

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<th>Table 1 P13K/Akt/mTOR pathway inhibitors currently in clinical development in advanced prostate cancer.</th>
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ADT, androgen deprivation therapy; CRPC, castration-resistant prostate cancer; HDAC, histone deacetylase inhibitor; MEK, mitogen-activated protein/extracellular signal-related kinase; mTORC, mammalian target of rapamycin complex; VEGF, vascular endothelial growth factor.
tumorigenesis (Jia et al. 2008). Similarly, the activity of MLN1117 was much lower in cells with PTEN deficiency (Jessen et al. 2011). Therefore, β-specific inhibitors such as GSK2636771 are in early-stage development, and it is likely that the efficacy of these inhibitors will depend on whether tumorigenesis is driven by PIK3CA mutation or loss of PTEN. A potential limitation of isoform-specific inhibition is the possibility of functional redundancy between isoforms. In PC-3 cells, for example, the combination of an α/δ PI3K inhibitor with a β-specific inhibitor (TGX-221) resulted in increased suppression of the pathway compared with inhibition of α/δ and β isoforms independently (Edgar et al. 2010).

Dual PI3K/TORC1/2 inhibitors

Dual PI3K/TORC1/2 inhibitors are ATP-competitive inhibitors that target all four p110 isoforms and both mTOR complexes and should lead to a more complete blockade of the PI3K/Akt/mTOR pathway. Indeed, the dual PI3K/TORC1/2 inhibitors GDC-0980 and BEZ235 inhibited PI3K/Akt/mTOR signaling and induced G1 arrest across a broad panel of cancer cell lines (Maira et al. 2008, Wallin et al. 2011). Greatest sensitivity to GDC-0981 was observed in breast, prostate, and lung cancer cells, and in contrast to the TORC1 inhibitors, GDC-0980 was also shown to induce apoptosis in cells with direct pathway activation via PIK3CA mutation or PTEN loss (Wallin et al. 2011). Furthermore, the effects of the dual PI3K/TORC1/2 inhibitor PI-101 on PC-3 cells were enhanced through combination with rapamycin or everolimus, as indicated by phosphorylated-Akt levels in vitro and tumor growth in vivo, suggesting that combining competitive and allosteric inhibition leads to improved suppression of the pathway (Mazzoletti et al. 2011). In the clinic, GDC-0980 and BEZ235 have been well tolerated in patients with solid tumors, with common adverse events including nausea, vomiting, diarrhea, and fatigue (Burriss et al. 2010, Wagner et al. 2011). With BEZ235, partial responses were observed in two patients (4%), and 14/51 (27%) of evaluable patients had stable disease for at least 4 months (Burriss et al. 2010). Issues surrounding the formulation of BEZ235 have delayed drug development to date for this agent, which remains in phase I testing at present. Phase I/II clinical trials of GDC-0980 and BEZ235 are ongoing in metastatic CRPC, alone and in combination with abiraterone acetate, as is a combination of BEZ235 with everolimus in advanced solid tumors (Table 1).

Akt inhibitors

As an important central regulator of PI3K/Akt/mTOR signaling, Akt has been a long-standing focus for therapeutic inhibition, but due to the homology between Akt and other kinases, Akt-selective drug development has been difficult. Preclinical studies with the allosteric Akt inhibitor perifosine have shown that it can induce cell cycle arrest and cell death in both PC-3 (Floryk & Thompson 2008) and PTEN-deficient CaP cells (Festuccia et al. 2008). However, despite a good safety profile in early clinical trials (the most common adverse events were diarrhea, nausea, fatigue, and vomiting), no evidence of radiographic or PSA response (> 50% decline in PSA) was observed in patients with CRPC (Posadas et al. 2005, Chee et al. 2007). More recently, ATP-competitive selective Akt inhibitors such as GSK690693 and GDC-0068 have demonstrated antitumor activity in prostate cancer xenograft models (Rhodes et al. 2008, Lin 2011). It remains to be seen whether the ATP-competitive Akt inhibitors will have clinical benefit, but a lack of clinical efficacy of perifosine suggests that Akt-independent PI3K signaling may be important in prostate cancer. For example, it has been shown that tumors with PIK3CA mutations rely on the Akt-independent target SGK3 for tumorigenesis (Vasudevan et al. 2009), suggesting that targeting of PI3K may be more effective than targeting Akt in some tumors. Alternatively, dosing of perifosine may not have led to sufficient inhibition of key Akt effectors, such as the eIF–4E complex. These results suggest the need for careful pharmacokinetic–pharmacodynamic studies in patients to determine optimal dosing and resistance mechanisms.

Combining PI3K/Akt/mTOR pathway inhibitors with other therapies

Similar to complex infectious diseases such as tuberculosis and HIV/AIDS, in which combination approaches revolutionized therapy and transformed a life-threatening illness into a curable or controllable illness with an excellent long-term prognosis, combination therapies, based on knowledge of feedback resistance pathways inherent to the cancer cell and the tumor microenvironment that are activated during single-agent therapy, are an emerging and necessary step in oncology (Glickman & Sawyers 2012). With PI3K/Akt/mTOR pathway inhibitors, use of combination partners may be particularly important due to the large degree of cross talk and reciprocal feedback regulation between the PI3K/Akt/mTOR pathway and other signaling pathways highlighted earlier.
Combination strategies in preclinical models have turned previously cytostatic activity into cytocidal activity and more durable remissions.

**Combination with anti-androgen therapy**

The clinical results of the aromatase inhibitor exemestane in combination with everolimus in metastatic estrogen receptor/progesterone receptor-positive breast cancer are testament to the power of rationale combination approaches (Baselga et al. 2012). Similar to the AR signaling pathway in prostate cancer, in breast cancer, there is evidence that the estrogen receptor signaling pathway interacts with the PI3K/Akt/mTOR pathway. Specifically, phosphorylation of the estrogen receptor by S6K1, downstream of TORC1, leads to ligand-independent signaling and may be a mechanism of resistance to endocrine therapy (Yamnik et al. 2009). In a large phase III study of patients with breast cancer who had progressed on an aromatase inhibitor, the addition of the mTOR inhibitor everolimus to exemestane resulted in a 6-month improvement in progression-free survival (Baselga et al. 2012). Given the known cross talk between the AR and PI3K/Akt/mTOR pathways (Carver et al. 2011), this study provides a rationale for combined AR and PI3K/Akt/mTOR targeted therapy and suggests that a clinical benefit with a combination approach may be possible for patients with prostate cancer as well. In support of this, combined targeting of the AR and PI3K/Akt/mTOR pathways with the PI3K inhibitor BEZ235 and the AR signaling inhibitor enzalutamide in PTEN-deficient cell lines resulted in a profound increase in apoptotic cell death, while only modest cytostatic activity was observed with each agent individually (Carver et al. 2011). Furthermore, combination of the mTOR inhibitor rapamycin with either chemical or surgical castration resulted in additive antitumor effects in a PTEN-deficient prostate cancer mouse model (Zhang et al. 2009), and the addition of everolimus to bicalutamide in a xenograft model harboring LN-CaP cells significantly reduced tumor growth rates compared with bicalutamide alone (Schayowitz et al. 2010). However, clinical results of the combination of the anti-androgen bicalutamide with the mTOR inhibitor everolimus in patients with CRPC have been conflicting. In a phase II trial, low activity was observed: two (6%) patients had a confirmed PSA response (≥50% reduction in PSA) and median time to progression was 8.7 weeks (Nakabayashi et al. 2012). Meanwhile, in a phase I/II study, partial responses (≥30% decline in PSA) were observed in nine patients (69%) compared with only one patient (20%) in the placebo-plus-bicalutamide arm (Pan et al. 2012). These variable results may be due to incomplete suppression of the PI3K/Akt/mTOR pathway with everolimus or due to inadequate activity of bicalutamide in patients with CRPC who have amplification of AR in their tumors (Chen et al. 2004). Further evaluation of four patients who responded to treatment vs four patients who did not revealed that responders had significantly elevated HER3 levels (Pan et al. 2012), and HER3 levels are now being investigated as a predictive biomarker for response to everolimus in hormone-treated prostate cancer patients. Combination studies of PI3K/Akt/mTOR pathway inhibitors with novel androgen synthesis inhibitors, such as abiraterone acetate or orteronel, or with novel anti-androgens, such as enzalutamide or ARN-509, may be more effective and are eagerly awaited. Currently, there are phase II studies investigating the combination of BKM120 and BEZ235 with abiraterone acetate (Table 1).

**Combination with chemotherapy**

Activation of the PI3K/Akt/mTOR pathway has recently been implicated in resistance to docetaxel (Qian et al. 2010), and combining PI3K/Akt/mTOR pathway inhibitors with docetaxel has demonstrated enhanced activity in a variety of preclinical models (Morgan et al. 2008, Fung et al. 2009, Dubrovská et al. 2010, Qian et al. 2010, Maira et al. 2012b, Morikawa et al. 2012). The enhanced efficacy of BEZ235 with docetaxel was shown to be a result of BEZ235-induced reduction of CD133+/CD44+ tumor progenitor cells, and docetaxel-induced reduction of the tumor bulk, which resulted in near complete tumor regression in a mouse prostate cancer xenograft model (Dubrovská et al. 2010). Phase I/II clinical trials of everolimus or temsirolimus in combination with docetaxel in CRPC have been completed and results are anticipated shortly. Phase I trials of BKM120 or GDC-0068 in combination with docetaxel in advanced solid tumors are ongoing.

**Combination with MEK inhibitors**

As activation of the RAS/RAF/MEK pathway has been implicated in conferring resistance to PI3K/Akt/mTOR pathway inhibition (Ihle et al. 2009), and significant activation of this pathway has been observed in both primary and metastatic prostate cancer (Mulholland et al. 2012), the combination of PI3K/Akt/mTOR inhibitors with inhibitors of the RAS/RAF/MEK pathway is a rationale approach. The combination of the mTOR inhibitor rapamycin with the MEK inhibitor PD325901 resulted in
synergistic growth inhibition in the androgen-responsive cell lines CWR22Rv1 (Gioeli et al. 2011) and CASP 2.1 (Kinkade et al. 2008) and the androgen-independent cell line CASP 1.1 (Kinkade et al. 2008). While in vivo, the combination displayed potent antitumorigenic activity in a mouse model of CRPC (Gioeli et al. 2011). In a phase I/II study in patients with advanced solid tumors, the combination of the MEK inhibitor GDC-0973 and the PI3K inhibitor GDC-0941 was shown to be well tolerated with a safety profile similar to that observed with either agent alone. Furthermore, decreases in RECIST-measurable target lesions were observed in five patients: two patients with melanoma (−75 and −25%); one patient with prostate cancer (−21%) and two patients with non-small-cell lung cancer (−18 and −13%) (Shapiro et al. 2011). In preclinical prostate cancer model systems driven by PTEN loss and RAS pathway activation, the inhibition of MEK reduced invasion and metastasis and reverted EMT, suggesting the importance of RAS pathway activation to invasiveness in prostate cancer (Mulholland et al. 2012).

Combination with RTK-targeted therapies

The EGFR family tyrosine kinases, including HER2 and HER3, play a role in the growth and survival of prostate cancer cells (Renner et al. 2008), and modulation of AR function by HER2/3 signaling has been documented (Mellinghoff et al. 2004). Specifically, HER2/3 signaling is required for AR function at reduced androgen concentrations (similar to concentrations achieved during androgen deprivation therapy). Under these conditions, HER2/3 increases binding of the AR to androgen response elements in the promoters of AR target genes and stabilizes the AR protein. To date, however, EGFR and HER-targeted therapies have not demonstrated significant clinical efficacy in prostate cancer even as single agents (Ziada et al. 2004, Small et al. 2007, Pezaro et al. 2009, Sridhar et al. 2010, Whang et al. 2011) or in combination with docetaxel (Gross et al. 2007), likely in part because PTEN loss is known to mediate resistance to EGFR family tyrosine kinase inhibitors (TKIs; Mellinghoff et al. 2007), or due to the lack of upregulation of these receptors when AR is not sufficiently blocked. This, combined with the fact that mTOR inhibition relieves negative feedback inhibition on and so upregulates HER2/3 signaling (Rodrik-Outmezguine et al. 2011), provides a robust rationale for co-targeting of RTKs and the PI3K/Akt/mTOR and/or AR pathways in prostate cancer. In preclinical studies, the combination of EGFR and Akt inhibition had synergistic anticancer activity in PTEN-deficient prostate cancer cells (Festuccia et al. 2008), while the dual EGFR and vascular endothelial growth factor (VEGF) TKI AEE788 combined with the mTOR inhibitor everolimus profoundly reduced tumor-endothelium and tumor-matrix contacts and suppressed cell growth in a variety of prostate cancer cells (Wedel et al. 2011a). Moreover, the triple combination of everolimus, AEE788, and the histone deacetylase inhibitor valproic acid demonstrated enhanced anticancer activity compared with each agent alone in PC-3, LN-CaP, and Du-145 cells (Wedel et al. 2011b). In the clinical setting, early results from a phase I/II clinical trial of cixutumumab, an anti-IGF-1R monoclonal antibody, in combination with temsirolimus in patients with chemotherapy-naïve metastatic CRPC have been encouraging; the combination was well tolerated, and for the seven evaluable patients, median time to progression was 32 weeks, with a range of 11–60 weeks (Rathkopf et al. 2011).

Future clinical perspectives

It is clear from autopsy studies of men with lethal prostate cancer that pathway-activating genomic aberrations in the PI3K pathway occur with a near 100% prevalence. This is accompanied by frequent aberrations in AR signaling and RAS pathway signaling, illustrating that mutational activation of PI3K does not exist in isolation and requires partners to create a lethal genotype/phenotype. Preclinical studies and early clinical data support the clinical investigation of PI3K/Akt/mTOR pathway inhibitors in combination with other active systemic agents in patients with prostate cancer. Recently, the novel androgen-depriving agents abiraterone acetate and enzalutamide have been shown to improve survival of patients with prostate cancer, even after the emergence of castration resistance (de Bono et al. 2011, Scher et al. 2012). Combining these therapies with PI3K/Akt/mTOR inhibitors may further enhance their clinical activity and/or reverse de novo and acquired resistance. Given the likely rapid emergence of resistance to single-agent therapies, initiating therapy with combination approaches would be the preferred route of clinical investigation. Conversely, in order to minimize cost or toxicity with long-term combination approaches, the sequential addition of PI3K inhibition to AR pathway inhibitors upon progression is also worthy of study.

A careful analysis of resistance and progression mechanisms in the clinical setting is required through a systematic analysis of circulating and tumor tissue biomarkers relevant to key oncogenic pathways such as...
AR splice variants, RAS pathway activation, and other key oncogenic drivers such as EMT and stemness pathways.

Another important clinical question is the predictive nature of PI3K/Akt/mTOR pathway activation and response to treatment. While there is a wealth of preclinical data implicating the activation of the PI3K/Akt/mTOR pathway in resistance development and enhanced sensitivity to PI3K/Akt/mTOR pathway inhibitors, the clinical validation of the predictive nature of PI3K/Akt/mTOR pathway alterations has been complicated. Obtaining tumor samples for molecular diagnostics is often impractical, and the range of aberrancies in this pathway, including kinase activation and phosphatase inactivation, is complex. Ideally, tumor sampling would occur each time a new treatment is considered, as archival tumor tissue from localized disease may not capture the acquisition of oncogenic events that lead to recurrence and tumor heterogeneity (Gerlinger et al. 2012). To this end, circulating tumor cells (CTCs) and CTC biomarkers may provide an opportunity for the relatively noninvasive molecular assessment of a patient’s tumor. AR amplification (Shaffer et al. 2007, Attard et al. 2009, Leversha et al. 2009) and PTEN loss (Attard et al. 2009) have been identified in the CTCs of patients with metastatic CRPC, highlighting the potential for the use of CTCs in biomarker-driven therapy decisions. It should be noted, however, that the only Food and Drug Administration-cleared CTC detection method, CellSearch (Veridex), fails to identify CTCs in as many as 50% of chemotherapy-naïve men with progressive metastatic disease (Dash et al. 2002, de Bono et al. 2008, Scher et al. 2011). Furthermore, due to the use of an epithelial biomarker to capture cells, the CellSearch system is unable to identify cells that have undergone EMT or developed stem cell-like properties (Armstrong et al. 2011). Therefore, improvements in the detection of CTCs may first be necessary before such diagnostic tools can reliably be used for molecular diagnosis of PI3K/Akt/mTOR pathway activation. Technologies that allow for the high-throughput sequencing of CTCs or circulating tumor DNA, RNA, or microRNA are emerging that may permit this personalized biomarker-driven precision algorithm to proceed more rationally (Diaz et al. 2012).

Conclusions

The high prevalence of PI3K/Akt/mTOR pathway alterations in prostate cancer, combined with evidence for the involvement of this pathway in the development of castration-resistant disease, has led to the intensive investigation of PI3K/Akt/mTOR pathway inhibitors in CRPC. While several inhibitors have demonstrated antitumor activity in preclinical models, cross talk and feedback inhibition along parallel pathways may mean that the success of these inhibitors will be dependent on their combination with other targeted or conventional therapies. Clinical trials investigating PI3K/Akt/mTOR pathway inhibitors as single agents and in combination are ongoing in CRPC, and investments are needed to determine predictive biomarkers and the early and late pharmacodynamic effects of these therapies in the clinic.

Declaration of interest

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