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Growth factor and signaling pathways and their relevance to prostate cancer therapeutics

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Abstract

Treatments that target the androgen axis represent an effective strategy for patients with advanced prostate cancer, but the disease remains incurable and new therapeutic approaches are necessary. Significant advances have recently occurred in our understanding of the growth factor and signaling pathways that are active in prostate cancer. In conjunction with this, many new targeted therapies with sound pre-clinical rationale have entered clinical development and are being tested in men with castration-resistant prostate cancer. Some of the most relevant pathways currently being exploited for therapeutic gain are HGF/c-Met signaling, the PI3K/AKT/mTOR pathway, Hedgehog signaling, the endothelin axis, Src kinase signaling, the IGF pathway, and angiogenesis. Here, we summarize the biological basis for the use of selected targeted agents and the results from available clinical trials of these drugs in men with prostate cancer.

Keywords

Prostate cancer; Angiogenesis; mTOR; c-Met; Hedgehog pathway; Insulin-like growth factor pathway

1 Introduction

Prostate cancer is the most commonly diagnosed non-cutaneous malignancy in the USA and is the second-leading cause of cancer-related death in men [1]. The introduction of screening with prostate-specific antigen (PSA) has improved the detection of early stage disease and has decreased prostate cancer mortality. However, many men still die of metastatic disease. In recurrent or advanced prostate cancer, the initial treatment involves androgen deprivation therapy, typically using a gonadotropin releasing hormone agonist with or without an androgen receptor antagonist. Although the majority of patients initially respond to androgen deprivation therapy, inevitably, there is progression of disease despite

maintenance of castrate levels of testosterone. In the past 5 years, four new FDA-approved therapies (abiraterone, sipuleucel-T, cabazitaxel, and enzalutamide) have been shown to extend survival in patients with metastatic castration-resistant prostate cancer (CRPC). At the same time, a deeper understanding of the growth factor and signaling pathways driving the malignant behavior of metastatic CRPC has led to the development of several targeted therapies in various stages of clinical testing (Fig. 1). This review will focus on the pathways and emerging therapies that have attracted the greatest attention in recent years, with an emphasis on agents that have reached phase II and III testing.

2 Targeting angiogenesis

Therapeutic strategies aimed at preventing the growth of new blood vessels to supply tumors have yielded clinical benefits for patients with many different types of cancers, most notably renal cell carcinoma. There is a strong preclinical basis for studying inhibitors of angiogenesis in prostate cancer, as this process appears to play an important role in prostate carcinogenesis and maintenance. One of the key factors in angiogenesis is hypoxia-induced factor-1 α (HIF-1 α), a transcription factor whose expression is regulated by oxygen levels and growth factor signaling. HIF-1 α controls expression of various genes including many that are involved in angiogenesis, such as vascular endothelial growth factor (VEGF). VEGF acts directly on endothelial cells to stimulate proliferation and to increase vascular permeability, forming the matrix upon which neovascularization can occur. In this manner, both hypoxia-dependent and hypoxia-independent mechanisms can induce angiogenesis [2]. In prostate cancer, neovascularization is not only triggered by the hypoxic tumor microenvironment, but also by aberrant growth factor signaling. For example, prostate cancer cells may aberrantly express both VEGF and the VEGF receptor (VEGFR). This suggests a dual role for this pathway with both paracrine signaling mechanisms that promote angiogenesis as well as autocrine signaling mechanisms that stimulate cell growth and proliferation [3, 4].

Many drugs that inhibit VEGF signaling have been tested in prostate cancer, including several that have been FDA-approved for the treatment of other solid tumors. The most well-known is bevacizumab, a humanized monoclonal antibody to VEGF. Bevacizumab was evaluated in a phase III cooperative group trial in patients with metastatic CRPC. Participants were treated with docetaxel and prednisone and either bevacizumab (15 mg/kg IV every 21 days) or placebo. Over 1,000 patients enrolled in the study. Progression-free survival (PFS) was improved in the group that received bevacizumab (9.9 versus 7.5 months, $p<0.001$), but overall survival did not differ significantly (22.6 versus 21.5 months, $p<0.18$) [5]. Grade 3 or higher toxicities were more common in the bevacizumab-treated arm, as was treatment-related mortality. Serious adverse events attributed to treatment with bevacizumab included hypertension, GI hemorrhage, and perforation, mucositis, and pneumonitis. Potential reasons for why the PFS benefit failed to translate into improved overall survival in this trial have been debated. The study authors speculate that the relative frequency of co-morbid conditions across the two study arms was imbalanced in favor of the placebo group. Moreover, the elderly population studied in this trial may have been predisposed to increased toxicity from VEGF-targeted therapy. Finally, the constraints of the trial design may have prevented the emergence of an overall survival benefit, because the

study was not prepared to examine the value of maintenance bevacizumab. Although patients were permitted to continue on single-agent bevacizumab or placebo if docetaxel was not tolerated, few patients did so. In other solid tumors, such as metastatic lung adenocarcinoma, continued treatment with bevacizumab following a fixed number of cycles of cytotoxic chemotherapy has translated into a modest survival advantage [5, 6]. Perhaps because of these clinical trial design issues that have clouded the results of the phase III study, investigators have continued to study the efficacy of bevacizumab in prostate cancer. Ongoing studies are evaluating its role in conjunction with a short course of androgen deprivation therapy in the PSA-recurrent/non-metastatic setting. It is also being studied in metastatic CRPC patients in combination with the mammalian target of rapamycin (mTOR) inhibitors everolimus and temsirolimus (Table 1).

An alternative strategy has emerged for targeting VEGF signaling using the VEGF-trap molecule aflibercept. Aflibercept is a cleverly designed decoy receptor that binds circulating VEGF ligand, thereby preventing its association with cellular VEGF receptors. The drug was studied in a multinational phase III trial in symptomatic metastatic CRPC patients. Over 1,200 patients were randomized to receive docetaxel plus either aflibercept or placebo. In this trial, there were no significant differences in progression-free or overall survival, and toxicities were higher in the aflibercept arm [7]. The increase in toxicities in the interventional arm mimicked the higher rate of adverse events with the docetaxel-bevacizumab combination. Due to these negative findings, no further studies of aflibercept are planned in patients with prostate cancer.

Attempts to target VEGF signaling with small molecule inhibitors in men with prostate cancer have shown some promise in phase II studies, but only one of these agents (sunitinib) has entered phase III testing. Sunitinib is a promiscuous tyrosine kinase inhibitor (TKI) that blocks VEGFR2 and platelet-derived growth factor- β signaling (PDGFR- β). A phase III study was conducted in patients with metastatic CRPC who progressed after receiving docetaxel chemotherapy. In this trial, over 800 men were randomized to single-agent sunitinib or placebo. While progression-free survival was superior for sunitinib (5.6 versus 3.7 months, $p=0.008$), there was no significant difference in overall survival compared to placebo (13.1 versus 12.8 months, $p=0.58$) [8]. The results of this study also beg the question of whether an overall survival (OS) benefit may have been observed if sunitinib had been continued beyond radiographic progression in patients who were tolerating the drug well.

Sorafenib, another multi-kinase inhibitor that can inhibit VEGFR, has also been tested in phase II trials in metastatic CRPC. Moderate activity was observed, but definitive phase III studies have not been launched and none are planned [9, 10]. Similar results were observed with cediranib (formerly AZD2171), a TKI that is more selective for VEGFR and also blocks activity of c-kit. In a single-arm, open-label phase II study that enrolled a heavily pre-treated population of metastatic CRPC patients, the median PFS and OS for patients receiving cediranib were 3.7 and 10.1 months, respectively. Although it is difficult to make cross-study comparisons, the median survival with cediranib was less than what was observed in the pivotal phase III trials that led to the approvals of cabazitaxel (15.1 months) and abiraterone (14.8 months) for metastatic CRPC patients in the post-docetaxel setting

[11]. Preliminary results from a phase II placebo-controlled study of docetaxel and cediranib have also been reported. In this trial, 58 participants were treated with docetaxel and prednisone and randomized to receive either cediranib or placebo. Toxicities were more pronounced in the cediranib group, and grade-3 or higher adverse events included neutropenia, fatigue, hypertension, anemia, diarrhea, and venous thromboembolism. Almost 70 % of the patients enrolled in the cediranib arm required a reduction in cediranib dosing. Although the PFS data are not mature, the investigators reported a higher partial response rate with cediranib (53 versus 33 %) in the 24 patients evaluable [12]. Importantly, while none of the trials using anti-angiogenesis agents in prostate cancer have shown a clinical benefit across a broad patient population, all studies involving small molecule inhibitors of VEGFR signaling have demonstrated that there are some patients in whom these drugs are certainly active. However, in the absence of a predictive biomarker, it is unlikely that sorafenib or cediranib will prove useful in the treatment of unselected patients with metastatic CRPC. Nevertheless, clinical trials investigating these and other small molecule VEGFR inhibitors are actively enrolling patients with advanced prostate cancer (Table 1).

Thalidomide and its derivative lenalidomide have also been tested in prostate cancer. The precise mechanism of action of these drugs is unclear, but they are believed to possess immunomodulatory properties and may also reduce neovascularization and inflammation in the tumor microenvironment [13]. Early phase studies in prostate cancer suggested a modest clinical benefit with thalidomide, but the largest studies have focused on lenalidomide because of its more favorable side effect profile. Phase I/II studies of lenalidomide suggested that it was efficacious in metastatic CRPC, as a reasonable percentage of patients experienced partial radiographic responses and a greater number had PSA declines [14, 15]. These encouraging results led to the design of an international phase III study to examine the efficacy of lenalidomide in conjunction with chemotherapy. Over 1,000 men were randomized to treatment with docetaxel plus either lenalidomide or placebo. However, the study was terminated early when it became apparent that survival was inferior for patients who received lenalidomide in combination with docetaxel. At the time of interim analysis, the median overall survival for the lenalidomide-docetaxel arm was 19.5 months, but had not yet been reached in the control arm (hazard ratio, 1.53, $p=0.0017$). Toxicities were more frequent and severe in the lenalidomide arm and included neutropenia, diarrhea, and pulmonary embolism. Likely as a consequence of toxicity, patients in the lenalidomide arm received fewer cycles of chemotherapy [16]. Based on these findings, it is unlikely that lenalidomide will have a role in the treatment of patients with prostate cancer.

Despite the disappointing results from the aforementioned phase III trials, several drugs that target angiogenesis in novel ways remain in clinical development. Perhaps the most promising of these is tasquinimod, a second-generation quinolone-3-carboxamide analogue. This drug inhibits angiogenesis by preventing the upregulation of HIF-1 α and the resultant aberrant VEGF expression. It also appears to induce expression of an endogenous anti-angiogenesis factor, thrombospondin-1. Through an alternative or complementary mechanism of action, the drug also inhibits S100A9, a protein involved in differentiation and cell cycle progression. Inhibition of S100A9 also prevents recruitment of myeloid derived suppressor cells (MDSCs), which are important figures in the tumor

microenvironment. MDSCs may participate in immune escape and other mechanisms by which tumors evade destruction by the immune system [17].

A phase II study of tasquinimod was conducted in minimally symptomatic men with metastatic CRPC who had not received chemotherapy. The primary endpoint was the proportion of patients without disease progression at 6 months (excluding PSA progression). Over 200 men were assigned in a 2:1 randomization to receive tasquinimod or placebo. Fewer men were progression-free at 6 months in the placebo group than in the tasquinimod group (31 versus 63 %, $p<0.001$). Median PFS was 7.6 months with tasquinimod versus 3.3 months with placebo ($p=0.0042$). The drug had minimal effect on PSA kinetics and few men that were treated with tasquinimod had a significant reduction in PSA. Common side effects of tasquinimod were fatigue, nausea, constipation, and anorexia. Grade-3 and higher toxicities included asymptomatic elevations in the lipase and amylase levels, anemia, and venous thrombosis [18]. On the basis of these results, tasquinimod has advanced to a phase III trial that is enrolling patients with similar characteristics as those in the phase II study. This trial will use PFS and OS as co-primary endpoints, and has already completed an accrual of 1,200 men with chemotherapy-naïve CRPC (Table 1).

Trebananib (formerly AMG386) is another novel angiogenesis inhibitor being evaluated in prostate cancer. This drug is a first-in-class angiopoietin (Ang) antagonist. The angiopoietins are ligands for the Tie-2 receptor, an essential signaling molecule for blood vessel remodeling and endothelial cell activation [19]. The expression pattern of angiopoietin-1 and -2 was studied in normal and malignant prostate tissue. In normal prostate tissue, Ang-1 was highly expressed in the basal epithelium, whereas Ang-2 was only weakly expressed. By contrast, high-grade malignant prostatic epithelium showed intense Ang-2 expression, with weaker staining for Ang-1. This suggests that disruptions in the balance between Ang-1 and -2 may be associated with tumor progression [20]. Trebananib is a recombinant peptide-Fc fusion protein (“peptibody”) containing a peptide sequence that binds Ang-1 and -2, thereby blocking their interaction with the Tie2 receptor. By neutralizing Ang-1 and -2, trebananib is thought to reduce tumor angiogenesis. A phase I study was conducted to assess the safety of trebananib, in which 32 patients with various malignancies were enrolled. The most common toxicities of trebananib were fatigue, edema, and proteinuria. Few grade 3 or higher toxicities were seen, but one patient died of respiratory failure that was probably the result of progressive disease, although the possibility of trebananib as a contributing factor could not be ruled out. Other toxicities common to anti-VEGF therapies (e.g., hypertension, hemorrhage, and thromboembolism) were not observed with trebananib. Several patients demonstrated stable disease, although no patients with prostate cancer were enrolled in the study [21]. Nevertheless, a phase II study is currently evaluating the efficacy of trebananib in conjunction with abiraterone in men with metastatic CRPC who have not previously been treated with chemotherapy (Table 1).

3 Targeting c-Met signaling

The c-Met receptor tyrosine kinase has received considerable attention as a potential therapeutic target for many solid tumors, including prostate cancer. c-Met is the cell surface receptor for the hepatocyte growth factor (HGF; also known as scatter factor). In normal

tissues, HGF is produced by stromal cells and signaling through c-Met occurs largely via paracrine mechanisms. HGF/c-Met signaling is thought to be important for many physiologic processes including embryogenesis, organogenesis, angiogenesis, wound healing, and repair of organ damage [22, 23]. Activation of c-Met can lead to signaling via multiple signal transduction pathways, including Src kinase and the phosphatidylinositol 3-kinase (PI3K)/AKT/MTOR and Ras/Raf/MEK/ERK cascades. These pathways activate many cellular processes relevant to cancer, including proliferation, survival, and resistance to apoptosis. HGF/c-Met signaling also promotes invasiveness, motility, and metastasis through changes in the structure of the cytoskeleton and altered integrin expression [24].

Abnormal c-Met expression has been observed in a variety of human malignancies, including prostate cancer. Mechanisms responsible for aberrant c-Met signaling include gene amplification and chromosomal rearrangement. Activating mutations and alternative splice variants can also lead to overactive c-Met signaling in cancer [23–25]. In prostate cancer, paracrine mechanisms are believed to be predominantly responsible for increased c-Met signaling [26]. High c-Met expression exists in approximately 50 % of primary prostate tumors at diagnosis and has been universally observed in bone metastases [27]. *In vitro*, many castration-resistant prostate cancer cell lines also express high levels of c-Met mRNA and protein and are responsive to HGF in a concentration-dependent manner [26]. Therefore, the relationship between the androgen receptor (AR) signaling and c-Met expression has been investigated. The AR appears to negatively regulate c-Met expression by interfering with Sp1, a transcription factor that binds to the promoter region of the c-Met gene and induces transcription. In support of this hypothesis, high c-Met expression has been observed in castration-resistant xenograft models. These findings have led to the conclusion that expression of c-Met and signaling via the HGF/c-Met axis may be important for the progression of prostate cancer to the castration-resistant state [28].

The presumed importance of c-Met signaling in prostate cancer and its widespread expression in osseous metastases has led investigators to study inhibitors of this signaling pathway in patients with advanced prostate cancer. The first attempt to target c-Met in prostate cancer utilized a monoclonal antibody against human HGF, rilotumumab. In a randomized phase II study, 144 patients with metastatic CRPC who had progressed after docetaxel chemotherapy were treated with mitoxantrone plus rilotumumab or placebo. Although rilotumumab was well tolerated, there were no differences in progression-free or overall survival between the two arms. Correlative studies demonstrated that patients with high levels of c-Met expression in archival tumor specimens showed a trend towards inferior outcomes [29]. Although monoclonal antibody-based strategies may prove successful in other tumor types or against other targets, in prostate cancer, the current approach is to inhibit HGF/c-Met signaling with small-molecule tyrosine kinase inhibitors.

Cabozantinib (XL184) is the most promising c-Met inhibitor in clinical development for the treatment of prostate cancer. It is an oral tyrosine kinase inhibitor that potently inhibits c-Met and VEGFR2 as well as RET. The safety of cabozantinib was evaluated in a phase I trial that established the maximum tolerated dose (MTD) at 175 mg daily, although doses this high have not been used in phase II or III clinical trials; although most patients had some evidence of toxicity, side effects were manageable and included diarrhea, fatigue,

decreased appetite, and rash. The main dose-limiting toxicities (DLTs) of cabozantinib were palmar-plantar erythrodysesthesia (hand-foot syndrome), mucositis, and elevations of liver enzymes (AST and ALT) as well as lipase elevations. Clinical benefit was seen in a broad range of tumor types, particularly in patients with medullary thyroid cancer (presumably due to inhibition of RET signaling). No patients with prostate cancer were enrolled on this phase I trial [30].

On the basis of the responses seen in this phase I study, an international phase II randomized discontinuation trial was conducted in nine tumor types simultaneously, including a cohort of men with metastatic CRPC. The dose selected for testing in this phase II study was 100 mg daily. All patients received open-label treatment with cabozantinib during a 12-week lead-in stage, and the trial then planned to randomize patients with stable disease at 12 weeks to cabozantinib or placebo. Randomization after the lead-in stage was suspended by the study oversight committee after the accrual of 122 patients because unexpected improvements were seen on bone scans across multiple tumor types (including prostate cancer). At that point, 31 patients with CRPC had been randomized and were evaluable for progression-free survival. Median PFS with cabozantinib was 23.9 weeks, compared to 5.9 weeks for placebo [31]. Ultimately, the study enrolled a total of 171 patients with metastatic CRPC, almost half of whom had previously received chemotherapy. Although the partial response rate per radiographic criteria was only 5 % after 12 weeks of treatment, 75 % of patients had stable disease. Cabozantinib appeared particularly active in treating bone metastases, as 12 % of patients had complete resolution of disease on bone scan (assessed by independent review). Reductions in pain and narcotic use were noted in patients for whom follow-up data was available. Importantly, PSA changes did not correlate with the results seen on imaging studies or other signs of clinical benefit; some patients had rising PSA levels despite reductions in the size of soft-tissue lesions or bone metastases. The toxicity profile of cabozantinib in this study was similar to that reported in the phase I trial, although higher rates of grade 3 hypertension were seen [31].

Following these encouraging results, cabozantinib is now being studied in two phase III trials in men with metastatic CRPC with progressive disease following treatment with docetaxel and abiraterone or enzalutamide. The first study, CabOzantinib Met Inhibition CRPC Efficacy Trial (COMET)-1, is evaluating the efficacy of single-agent cabozantinib versus placebo, with a primary endpoint of overall survival. The second study, COMET-2, is investigating the effect of cabozantinib on quality-of-life measures and pain control, in comparison to mitoxantrone; the primary endpoint of COMET-2 is the frequency of durable pain responses at week 12. Meanwhile, a recently launched phase I study is evaluating the safety of combined treatment with cabozantinib and abiraterone in men with metastatic CRPC who have already received chemotherapy (Table 2).

Another small molecule inhibitor of c-Met, tivantinib (formerly ARQ197), is being studied in many solid tumors including prostate cancer. Tivantinib functions by stabilizing an inactive configuration of c-Met, thereby preventing downstream signaling. It does not compete with ATP for binding, a unique property compared to other c-Met inhibitors in development. The drug may also promote degradation of c-Met via the ubiquitin-proteasome pathway. The safety of tivantinib was evaluated in a phase I trial, where the main DLTs

were fatigue, mucositis, palmar-plantar erythrodysesthesia, hypokalemia, and neutropenia. Correlative pharmacodynamic studies assessed intratumoral phosphorylated and total c-Met levels, and both were found to decrease during treatment with tivantinib. Thirteen patients with metastatic CRPC were included in the phase I study, but no RECIST responses were seen [32]. The efficacy of tivantinib is now being evaluated in a dedicated phase II study of patients with asymptomatic or minimally symptomatic metastatic CRPC in the pre-chemotherapy setting (Table 2).

4 Targeting the PI3K/AKT/MTOR pathway

The PI3K/AKT/MTOR pathway is an important signaling cascade in many different types of human cancer. This pathway has been linked to cell survival, differentiation, proliferation, growth, metabolism, migration, and angiogenesis. Normally, signaling via this pathway begins with binding of a growth factor to a receptor tyrosine kinase resulting in downstream activation of PI3K. Alternatively, activation of PI3K can occur via Ras signaling and the G-protein-coupled receptors. PI3K phosphorylates its substrate, phosphatidylinositol 4,5-bisphosphate (PIP₂) to produce phosphatidylinositol 3,4,5-triphosphate (PIP₃). PIP₃ can proceed to bind to the pleckstrin homology domains of various signaling proteins and initiate downstream signaling via AKT. This pathway is negatively regulated by the protein tyrosine phosphatase and tensin homolog (PTEN), which dephosphorylates PIP₃ to PIP₂ thereby terminating further signaling [33, 34]. The PI3K/AKT signaling cascade promotes cell survival and resistance to apoptosis through several different mechanisms, including interactions with the Bcl-2 family members BAD and BAX, NF-kappa-B, and the p53 antagonist Mdm2. Also downstream of this pathway is the mTOR. Activation of mTOR leads to increased protein synthesis through phosphorylation of ribosomal proteins and translation elongation factors. In this fundamental way, mTOR is an important modulator of cell growth. Multiple feedback loops and regulators control mTOR signaling, and the complex integrates inputs from various metabolic, growth factor, and survival pathways [33–35].

Preclinical laboratory data has provided a compelling foundation for studying the role of inhibitors of PI3K and its downstream targets in prostate cancer. Taylor et al. performed genomic profiling of 218 primary or metastatic prostate cancers, integrating information gathered from assessment of DNA copy number, mRNA expression profiles, and focused exon resequencing. A core pathway analysis showed that altered signaling in the PI3K pathway was present in nearly half of all the primary prostate tumors and all of the prostate cancer metastases tested. Approximately 40 % of all cases demonstrated loss of function of PTEN through deletion, silencing mutation, or reduced expression. In contrast to many other cancers, activating mutations in the PIK3CA gene were rare. However, loss of function mutations in the regulatory subunits PIK3R1 and PIK3R3 were prevalent, suggesting another mechanism for constitutive activation of PI3K in prostate cancer [36].

Despite these revealing laboratory observations, attempts to target segments of the PI3K/AKT/mTOR signaling pathway in prostate cancer patients have been disappointing thus far. Studies of the mTOR inhibitors rapamycin, everolimus, and temsirolimus as single agents and in combination with the androgen receptor antagonist bicalutamide failed to

demonstrate clinical activity in metastatic CRPC [37–39]. Nevertheless, based on preclinical data that mTOR inhibition can reverse chemotherapy resistance in PTEN-deficient prostate cancer cell lines, ongoing trials are examining the efficacy of combined treatment with mTOR inhibitors and docetaxel [40, 41]. Other novel mTOR inhibitors and combination therapies are also under investigation (Table 3).

One possible explanation for the failure of single-agent mTOR inhibitors to show efficacy in prostate cancer is the hypothesis that mTOR blockade leads to feedback-driven up-regulation of signaling molecules upstream in the PI3K pathway. Seminal research by Carver et al. has demonstrated the existence of bidirectional cross-talk between the PI3K pathway and AR signaling. For example, in a preclinical model, inhibition of the PI3K pathway resulted in activation of AR signaling in PTEN-deficient prostate cancer cells. Similarly, the AR antagonist enzalutamide appeared to upregulate AKT signaling by reducing levels of the regulatory phosphatase PHLPP. Combined blockade with the dual PI3K/mTOR inhibitor, BEZ235, and enzalutamide led to reductions in tumor size in xenograft models of human prostate cancer [42]. This work provides a sound rationale for simultaneous targeting of both pathways.

Under this premise, BEZ235 is currently being studied in combination with abiraterone in men with advanced CRPC (Table 3). The first-in-human phase I study showed that this drug was tolerable, as no DLTs were observed at the doses tested. Frequently reported side effects were fatigue and gastrointestinal symptoms. A few tumor responses were seen in the phase I study, which enrolled patients with various solid malignancies. Patients whose tumors demonstrated activated PI3K pathway signaling were the most likely to respond to treatment with BEZ235. Because of pharmacokinetic variability, the drug was reformulated to improve bioavailability, which has delayed clinical development [43, 44].

Efforts to develop the potent and specific AKT inhibitor MK2206 are also seeking to capitalize on the preclinical observations that simultaneous AR blockade and PI3K pathway inhibition may be synergistic. Previous phase II studies of another putative inhibitor of AKT, perifosine, have been disappointing. However, correlative pharmacodynamic studies were not performed in perifosine-treated patients, and therefore, it is unclear whether target inhibition was actually achieved in these studies [45, 46]. Conversely, pharmacodynamic correlates to the phase I study that established the safety profile and MTD of MK2206 have confirmed its ability to target and inhibit AKT in humans. The most frequent side effects of MK2206 observed in the phase I study were hyperglycemia, nausea, and diarrhea. The DLTs were skin rash and stomatitis, and the recommended dose for phase II testing was 60 mg of MK2206 administered on alternate days [47]. It remains to be seen whether MK2206 will prove to be more efficacious than perifosine in the clinic. MK2206 is currently being investigated in conjunction with bicalutamide in a cooperative group trial enrolling men with PSA-recurrent/non-metastatic disease after failed local therapy (Table 3).

The pan-PI3K inhibitors BKM120 and PX-866 are also being tested in phase II trials of metastatic CRPC. Both of these drugs potently inhibit wild-type and mutant class I PI3K isoforms. Phase I studies have included very few patients with prostate cancer, although one man with metastatic CRPC that received PX-866 experienced prolonged stable disease.

Interestingly, although the two drugs purport the same mechanism of action, their side effect profiles are distinct. DLTs on the PX-866 phase I study were primarily gastrointestinal symptoms, including diarrhea and transaminitis. During the phase I study of BKM120, similar gastrointestinal symptoms were seen but the drug had additional toxicities not seen with PX-866 including rash, hyperglycemia, and neuropsychiatric effects such as mood alterations and depression [48, 49]. As phase II trials move forward (Table 3), attention to the correlative pharmacodynamic studies for these drugs is imperative.

5 Targeting hedgehog signaling

The evolutionary-conserved hedgehog (Hh) signaling pathway is well-known for its essential role in embryogenesis. Binding of one of the three Hh signaling ligands (Sonic, India, or Desert) to one of the Patched receptors relieves repression of Smoothened, a G-protein-coupled receptor that initiates the signaling cascade. The pathway culminates with activation of the GLI family of transcription factors [50]. In prostate cancer, there is evidence for hyperactivity of this pathway. For example, immunohistochemical staining of normal and malignant prostate tissue has shown that expression of GLI2 is higher in tumor cells than in benign prostatic epithelium, and expression of Sonic Hh and patched increases with tumor grade. A retrospective study examining the mRNA and protein expression of several Hh family members in prostatectomy specimens found that higher levels of expression correlated with poor prognostic features such as larger tumor size, higher pre-treatment PSA level, increased Gleason score, and advanced stage [51]. Unlike basal cell carcinoma and medulloblastoma, activating mutations in Hh pathway genes are rare or nonexistent in human prostate cancer, and most studies suggest that aberrant signaling occurs in a ligand-mediated paracrine fashion. Preclinical models suggest that inhibition of Hh signaling may have therapeutic efficacy in human prostate cancer [52–54]. This is also supported by an early phase study of the smoothened antagonist itraconazole in patients with metastatic CRPC.

The discovery that the antifungal drug, itraconazole, has the ability to inhibit Hh signaling was an unexpected one. Additionally, itraconazole also appears to inhibit angiogenesis through unknown mechanisms [55]. A randomized non-comparative phase II study of itraconazole was conducted in men with metastatic CRPC to assess the efficacy of two different doses of this agent (200 or 600 mg/day). The primary endpoint of the study was freedom-from-PSA progression at 6 months. While the low-dose arm closed early for futility, the rate of freedom-from-PSA progression at 6 months was 48 % in the high-dose arm. Median progression-free survival was 8.3 months. Common side effects of itraconazole were fatigue, nausea, constipation, and peripheral edema. Grade-3 toxicities included hypokalemia, hypertension, and rash. Correlative pharmacodynamic studies showed reduced expression of GLI1 in skin biopsies in two thirds of patients, an effect that was associated with improved freedom-from-PSA progression and progression-free survival [56]. Based on these encouraging results, another phase II study of itraconazole has been designed for men with PSA-recurrent/non-metastatic cancer after failure of local therapy (Table 4).

6 Targeting Src kinase signaling

Src is an intracellular non-receptor tyrosine kinase that interacts with various transmembrane receptors and participates in a multitude of signal transduction pathways that regulate cell proliferation, differentiation, survival, adhesion, migration, invasion, and angiogenesis. Src has also been implicated in bone metabolism and it may play a role in the development and maintenance of osseous metastases. In prostate cancer, Src appears to be involved in the transition to the castration-resistant phenotype. Inhibition of this pathway *in vitro* and *in vivo* has been shown to impede tumor growth in an androgen-independent model [57].

Dasatinib, an inhibitor of multiple tyrosine kinases including Src, suppressed growth of prostate cancer in cell lines and in a murine xenograft model [58]. Early phase studies in men with metastatic CRPC suggested that dasatinib may be efficacious in human prostate cancer [59, 60], and a phase III study was initiated. Over 1,500 men with metastatic CRPC undergoing treatment with docetaxel and prednisone were randomized to receive dasatinib 100-mg daily or placebo in addition to chemotherapy. Preliminary results of the study have been reported, showing no difference in overall survival or progression-free survival between the two study arms [61]. As an alternative strategy, a randomized phase II study examining the efficacy of dasatinib in combination with abiraterone is continuing to enroll patients with metastatic CRPC in the pre-chemotherapy setting (Table 5).

Saracatinib (formerly AZD0530) is another Src inhibitor that has been evaluated in advanced CRPC, in a nonrandomized single-arm phase II study of 28 patients. Although the drug was tolerable, only five patients had transient PSA responses. None had reductions of greater than 30 % of the baseline value. Grade 3 toxicities included elevated liver transaminases, nausea, vomiting, and lymphopenia [62]. A randomized, placebo-controlled phase II trial of saracatinib in men with metastatic CRPC previously treated with docetaxel is ongoing (Table 5). Another non-ATP-competitive Src inhibitor, KX2-391, did not show evidence of anti-tumor activity in men with metastatic CRPC in a single-arm phase II study, although several patients had post-treatment reductions in markers of bone metabolism [63].

7 Targeting the endothelin axis

The endothelins (ET-1, ET-2, and ET-3) are a class of small peptides that modulate vasoconstriction, nociception, cell proliferation, bone remodeling, and hormone production. In normal prostate tissue, ET-1 is produced by prostatic epithelial cells and signals through its receptor, ET_A. In prostate cancer, mechanisms for clearance of ET-1 are diminished and ET_A receptors are overexpressed. ET-1 is also produced and secreted by malignant cells leading to increased signaling through autocrine mechanisms. In addition to serving as a mitogenic stimulus, ET-1 may also be involved in tumor invasion by inducing the expression of matrix metalloproteinases that facilitate cell migration. Because ET-1 is a mitogen for osteoblasts and also decreases osteoclastic bone resorption, paracrine signaling between osteoblasts and prostate cancer cells may enhance the development of bone metastases [64].

Based on these observations, two selective ET_A receptor antagonists have been developed and tested in prostate cancer patients. Zibotentan (formerly ZD4054) was studied in two

randomized phase III clinical trials (the ENTHUSE program), both as a single agent and in combination with docetaxel in patients with metastatic CRPC. Both studies failed to show a survival benefit using zibotentan and there were no significant differences in the secondary endpoints of progression-free survival, pain response, and time-to-new-bone metastases [65, 66]. Zibotentan was also investigated in patients with PSA-recurrent/non-metastatic CRPC in a study and was terminated early at the time of a planned interim analysis due to an inability to meet the primary endpoint of superior overall survival [67]. Atrasentan has also been studied extensively in prostate cancer and failed to show efficacy [68–70]. Due to these disappointing results, further studies of agents modulating the endothelin axis are not planned for the treatment of prostate cancer.

8 Targeting the insulin-like growth factor pathway

The insulin-like growth factor 1 receptor (IGF-1R) is a receptor tyrosine kinase whose ligand is IGF-1, a single-chain polypeptide with sequence homology to insulin. Most IGF-1 is synthesized in the liver but several cancers have also been shown to aberrantly produce the polypeptide as well, suggesting that the IGF pathway may act through endocrine, paracrine, or autocrine signaling mechanisms. Binding of IGF-1 to the IGF-1R leads to phosphorylation of adaptor proteins that, in turn, leads to differential activation of multiple downstream signaling pathways, including the PI3K and the Ras/Raf/MEK/Erk signaling cascades. These intracellular signals affect a number of fundamental cellular functions, including apoptosis, cell growth, proliferation, and differentiation [71, 72].

Epidemiological studies have suggested that the IGF pathway may play a key role in prostate carcinogenesis. Several investigators have observed that individuals with high levels of plasma IGF-1 have an increased risk of developing prostate cancer [73, 74]. Laboratory research has also suggested a role for the IGF pathway in both androgen-sensitive and castration-resistant prostate cancers. An analysis of human tumors showed that the IGF-1R is overexpressed in castration-resistant tumors. Targeting the IGF-1R with a monoclonal antibody inhibited tumor growth in androgen-sensitive and castration-resistant xenograft models [75, 76]. The IGF pathway may also be involved in resistance to mTOR inhibition. As discussed earlier in this review, *in vitro* and *in vivo* models have demonstrated that mTOR blockade leads to feedback-driven upregulation of signaling molecules upstream in the PI3K pathway, particularly AKT. This occurs as a consequence of bidirectional cross-talk between the PI3K/AKT/mTOR pathway and other mediators of signal transduction, including the AR pathway and IGF-1R pathway. Work by O'Reilly et al. has shown that this feedback-driven activation of AKT is dependent on IGF-1R signaling. In this model, concomitant use of the mTOR inhibitor rapamycin and an IGF-1R monoclonal antibody or kinase inhibitor led to a significant reduction in phospho-AKT levels, compared to treatment with rapamycin alone. The same investigators also observed that IGF-1R inhibition sensitized cells to treatment with rapamycin by enhancing cell cycle arrest and induction of apoptosis [77]. Thus, simultaneous targeting of the IGF-1R and mTOR pathways may be an efficacious strategy.

Attempts to block IGF-1R signaling in prostate cancer patients have followed these observations. Monoclonal antibodies specific to the IGF-1R and small molecules that aim to

inhibit its tyrosine kinase activity have been developed. Preliminary results from phase II trials involving these agents have been reported. Cixutumumab (formerly IMC-A12) is a fully human IgG1 monoclonal antibody targeting the IGF-1R. A phase II study enrolled 31 asymptomatic men with meta-static CRPC who were treated with cixutumumab every 2 weeks until disease progression or intolerable toxicity. Nine patients experienced stable disease for greater than 6 months, and three patients had PSA reductions. The most common toxicities were fatigue and hyperglycemia; grade 3 or higher toxicities included thrombocytopenia, hyperkalemia, pneumonia, and leukoencephalopathy [78]. Cixutumumab has also been studied in combination with mitoxantrone in metastatic CRPC patients who progressed on docetaxel. PSA responses were reported in 18 % of patients, but progression-free survival was a disappointing 4.1 months [79]. Linsitinib (formerly OSI-906) is a small molecule inhibitor of the IGF-1 and insulin receptor that is being evaluated in metastatic CRPC in the pre-chemotherapy setting. Preliminary results from 18 patients with asymptomatic or minimally symptomatic disease enrolled on a phase II study have been reported. Although transient PSA declines were noted, the majority of patients discontinued therapy due to PSA progression after a median of 3 months of therapy. Notable side effects included fatigue, liver function test abnormalities, prolonged QT interval, nausea, and vomiting [80]. However, it is unclear whether the benefits that have been reported preliminarily in these early phase studies are significant enough to warrant larger clinical trials in the metastatic CRPC population. Moreover, the toxicities associated with targeting this pathway may be too severe to justify exploration in earlier-stage patients, for example, those with PSA-recurrent/non-metastatic prostate cancer. Finally, based on the available data from preclinical models, it may be worthwhile to consider dual inhibition of the IGF-1 pathway and PI3K/AKT/mTOR pathway. Several such studies are now in the preliminary stages of development (Table 6).

9 Concluding remarks

In the past 5 years, the therapeutic landscape for prostate cancer has undergone a significant evolution, with the approval of two new agents (abiraterone and enzalutamide) that have been shown to extend survival in patients with metastatic CRPC. Prostate cancer also has the notable distinction of garnering the approval of the first therapeutic cancer vaccine (sipuleucel-T) and a new chemotherapeutic (cabazitaxel). At the same time, our understanding of the growth factor and signaling pathways that are active in prostate cancer has expanded, as is highlighted in this review. Clinicians are now armed with this knowledge and charged with the task of ensuring that it is successfully translated into new and effective treatments for patients with this disease. As a consequence, many new targeted therapies with sound preclinical rationale have entered clinical development and are being tested in this patient population.

However, many potential pitfalls and challenges lie ahead. First, it is imperative that researchers design thoughtful correlative pharmacodynamic studies to accompany the early phase trials of these agents, to ensure that target inhibition is actually being achieved at doses that are tolerable for patients. Furthermore, we must utilize the information gathered from the laboratory to identify potential biomarkers that are predictive of response to these targeted therapies. Large-scale integrative genomics studies (such as the one performed by

Taylor et al. [36]) are a reasonable starting point for researchers interested in developing such hypotheses. The identification of predictive biomarkers has been essential to the development of targeted therapies in other cancers, such as non-small cell lung cancer, breast cancer, and melanoma. As we have seen with the VEGF-targeted therapies, there is a subset of CRPC patients in whom these agents are potentially active. But in the absence of a predictive biomarker, it will be impossible to identify such patients. Next, investigators must carefully consider the optimal setting in which these targeted therapies are most likely to be efficacious. Certainly, it is reasonable to study these agents in patients with refractory disease, where there is an unmet need for new therapies. However, investigators must recognize that stronger scientific rationale may support the use of some therapies earlier in the course of disease. For example, it is intriguing to consider the possibility of using inhibitors of c-MET in the PSA-recurrent/non-metastatic setting after local failure, because of the implications that the HGF/c-met signaling is involved in the progression of prostate cancer to a castration-resistant state. Finally, because of the existence of bidirectional cross-talk and reciprocal feedback loops between multiple pathways, thoughtful combinatorial strategies may be necessary to overcome resistance to monotherapies. Accordingly, simultaneous targeting of the AR or IGF-1R in conjunction with mTOR inhibition may represent an efficacious strategy.

As our understanding of the growth factor and signaling pathways driving the malignant behavior of prostate cancer has expanded, so too has the armamentarium of drugs available for clinical testing. The stage is set for the next evolution in prostate cancer treatment.

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Fig. 1. Signaling pathways and targets in prostate cancer. Abbreviations: Patched (*PTCH1*); sonic Hedgehog (*SHH*); smoothened (*SMO*); hepatocyte growth factor (*HGF*); insulin-like growth factor-1 (*IGF-1*); insulin-like growth factor-1 receptor (*IGF-1R*); vascular endothelial growth factor (*VEGF*); vascular endothelial growth factor receptor (*VEGFR*); receptor tyrosine kinase (*RTK*); thrombospondin-1 (*TSP-1*); angiopoietin (*ANG*); G-coupled protein (*G*); insulin receptor substrate (*IRS*); phosphatidylinositol 3-kinase (*PI3K*); phosphatidylinositol 4,5-bisphosphate (*PIP2*); phosphatidylinositol 3,4,5-triphosphate (*PIP3*); mammalian target of rapamycin (*mTOR*)

Table 1

Selected ongoing clinical trials of drugs targeting angiogenesis in prostate cancer, adapted from clinicaltrials.gov

Target	Agent	Phase	Summary	Identifier
VEGF	Bevacizumab	II	Randomized efficacy study; Short-course androgen deprivation therapy ± bevacizumab in PSA-recurrent/non-metastatic prostate cancer	NCT00776594
VEGF mTOR	Bevacizumab Everolimus	Ib/II	Dose-finding/efficacy study; Docetaxel + RAD001 (everolimus) and bevacizumab in metastatic CRPC	NCT00574769
VEGF mTOR	Bevacizumab Temozolomide	I/II	Dose-finding/efficacy study; Bevacizumab + temozolomide in metastatic CRPC	NCT01083368
VEGFR2 PDGFR-β Src kinase	Sunitinib Dasatinib	II	Randomized efficacy study; Abiraterone ± sunitinib (or dasatinib) in metastatic CRPC	NCT01254864
VEGFR1 VEGFR2 VEGFR3	Cediranib	II	Randomized efficacy study; Docetaxel ± cediranib in metastatic CRPC	NCT00527124
VEGFR1 VEGFR2 VEGFR3 Src kinase	Cediranib Dasatinib	II	Randomized efficacy study; Cediranib ± dasatinib in metastatic CRPC	NCT01260688
VEGFR1 VEGFR2 VEGFR3 PDGFR-β	Pazopanib	I/II	Dose-finding/efficacy study; Docetaxel ± pazopanib in metastatic CRPC	NCT01385228
S100A9 Thrombospondin-1	Tasquinimod	I	Dose-finding study; Cabazitaxel + tasquinimod in metastatic CRPC	NCT01513733
S100A9 Thrombospondin-1	Tasquinimod	III	Randomized efficacy study; Tasquinimod vs. placebo in metastatic CRPC	NCT01234311
Ang-1 Ang-2	Trebananib	II	Randomized efficacy study; Abiraterone ± trebananib metastatic CRPC	NCT01553188

VEGF vascular endothelial growth factor, *mTOR* mammalian target of rapamycin, *CRPC* castration-resistant prostate cancer, *VEGFR2* vascular endothelial growth factor receptor-2, *PDGFR-β* platelet-derived growth factor receptor-β, *VEGFR1* vascular endothelial growth factor receptor-1, *VEGFR3* vascular endothelial growth factor receptor-3, *Ang-1* angiopoietin-1, *Ang-2* angiopoietin-2

Table 2

Selected ongoing clinical trials of drugs targeting c-Met signaling in prostate cancer, adapted from clinicaltrials.gov

Target	Agent	Phase	Summary	Identifier
c-Met VEGFR2	Cabozantinib	III	Randomized efficacy study; Cabozantinib vs. placebo in metastatic CRPC	NCT01605227
c-Met VEGFR2	Cabozantinib	III	Randomized efficacy study; Cabozantinib vs. mitoxantrone in metastatic CRPC	NCT01522443
c-Met VEGFR2	Cabozantinib	I	Dose-finding study; Cabozantinib + abiraterone in metastatic CRPC	NCT01574937
c-Met VEGFR2	Cabozantinib	II	Single-arm efficacy study; Cabozantinib in metastatic CRPC	NCT01428219
c-Met	Tivantinib	II	Randomized efficacy study; Tivantinib vs. placebo in metastatic CRPC	NCT01519414

VEGFR2 vascular endothelial growth factor receptor-2, *CRPC* castration-resistant prostate cancer

Table 3

Selected ongoing clinical trials of drugs targeting the PI3K/AKT/mTOR pathway in prostate cancer, adapted from clinicaltrials.gov

Target	Agent	Phase	Summary	Identifier
mTOR VEGF	Everolimus Bevacizumab	Ib/II	Dose-finding/efficacy study; Docetaxel + RAD001 (everolimus) and bevacizumab in metastatic CRPC	NCT00574769
mTOR VEGF	Temsirolimus Bevacizumab	I/II	Dose-finding/efficacy study; Temsirolimus + bevacizumab in metastatic CRPC	NCT01083368
mTOR	Everolimus	I/II	Dose-finding/efficacy study; Docetaxel + RAD001 (everolimus) in metastatic CRPC	NCT00459186
mTOR IGF-1R	Temsirolimus Cixutumumab	I/II	Dose-finding/efficacy study; Temsirolimus + cixutumumab in metastatic CRPC	NCT01026623
mTOR AKT Notch	Ridaforolimus MK2206 MK0752	I	Dose-finding study; Ridaforolimus + MK2206 or MK0752 in solid tumors including metastatic CRPC	NCT01295632
PI3K mTOR	BEZ235	I/II	Dose-finding/efficacy study; Abiraterone + BEZ235 in metastatic CRPC	NCT01717898
PI3K mTOR	BEZ235 BKM120	Ib	Dose-finding study; Abiraterone + BEZ235 or BKM120 in CRPC	NCT01634061
AKT	MK2206	II	Randomized efficacy study; Bicalutamide ± MK2206 in PSA-recurrent/non-metastatic prostate cancer	NCT01251861
PI3K	BKM120	II	Single-arm efficacy study; BKM120 in metastatic CRPC	NCT01385293
PI3K	BKM120	Ib	Single-arm efficacy study; Abiraterone + BKM120 in metastatic CRPC	NCT01741753
PI3K	PX-866	II	Single-arm efficacy study; PX-866 in advanced CRPC	NCT01331083

mTOR mammalian target of rapamycin, *VEGF* vascular endothelial growth factor, *CRPC* castration-resistant prostate cancer, *IGF-1R* insulin-like growth factor-1 receptor, *PI3K* phosphatidylinositol 3-kinase

Table 4

Selected ongoing clinical trials of drugs targeting Hedgehog signaling in prostate cancer, adapted from clinicaltrials.gov

Target	Agent	Phase	Summary	Identifier
Smoothed (SMO)	Itraconazole	II	Single-arm efficacy study; Itraconazole in docetaxel-refractory metastatic CRPC	NCT01450683
Smoothed (SMO)	Itraconazole	II	Single-arm efficacy study; Itraconazole in PSA-recurrent/non-metastatic prostate cancer	NCT01787331

Table 5

Selected ongoing clinical trials of drugs targeting Src kinase signaling in prostate cancer, adapted from clinicaltrials.gov

Target	Agent	Phase	Summary	Identifier
Src kinase VEGFR1 VEGFR2 VEGFR3	Dasatinib Cediranib	II	Randomized efficacy study; Cediranib ± dasatinib in metastatic CRPC	NCT01260688
Src kinase	Dasatinib	III	Randomized efficacy study; Docetaxel ± dasatinib in metastatic CRPC	NCT00744497
Src kinase	Dasatinib	II	Randomized efficacy study; Abiraterone ± dasatinib in metastatic CRPC	NCT01685125
Src kinase VEGFR2 PDGFR-β	Dasatinib Sunitinib	II	Randomized efficacy study; Abiraterone ± dasatinib (or sunitinib) in metastatic CRPC	NCT01254864
Src kinase	Saracatinib	II	Randomized efficacy study; Saracatinib vs. placebo in metastatic CRPC	NCT01267266

VEGFR1 vascular endothelial growth factor receptor-1, *VEGFR2* vascular endothelial growth factor receptor-2, *VEGFR3* vascular endothelial growth factor receptor-3, *CRPC* castration-resistant prostate cancer, *PDGFR-β* platelet-derived growth factor receptor-β

Table 6

Selected ongoing clinical trials of drugs targeting the IGF pathway in prostate cancer, adapted from clinicaltrials.gov

Target	Agent	Phase	Summary	Identifier
IGF-1R mTOR	Cixutumumab Temozolimus	I/II	Dose-finding/efficacy study; Cixutumumab + temozolimus in metastatic CRPC	NCT01026623
IGF-1R	Cixutumumab	II	Single-arm efficacy study; Cixutumumab in metastatic CRPC	NCT00520481
IGF-1R	Linsitinib	II	Single-arm efficacy study; Linsitinib in metastatic CRPC	NCT01533246

IGF-1R insulin-like growth factor-1 receptor, *mTOR* mammalian target of rapamycin, *CRPC* castration-resistant prostate cancer