

Evolving therapeutic concepts in prostate cancer based on genome-wide analyses (Review)

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Abstract. Treatment of castration resistant prostate cancer (CRPC) continues to represent a major urooncological challenge due to tumor heterogeneity and the inevitable development of therapy resistance. Although androgen deprivation therapy retains an important role in the management of CRPC, recent evidence suggests that a broader spectrum of therapeutic targets may improve patient response and delay development of advanced disease. Genome-wide analyses have identified four major signaling nodes that are most frequently altered in prostate cancer: i) the androgen receptor (AR); ii) the PI3K pathway; iii) the Ras/Raf/MEK/ERK pathway; and iv) the retinoblastoma protein (pRB) signaling pathway. Extensive crosstalk and redundancy exists between these signaling pathways, which underscores the need for combination therapies. There are several novel AR pathway inhibitors currently in clinical use. Clinical trials are being performed on single-agent PI3K inhibitors with some success in tumors with genetically altered PI3K components. MEK/ERK inhibitors are also in clinical trials and the importance of pRB inactivation in prostate cancer is becoming more widely recognized. A greater understanding of the effects of single agent therapy on compensatory signaling pathway activation that can potentially thwart antitumoral responses is urgently needed and will provide additional insight into the mechanism of therapy resistance and how to further delay the progression to lethal disease.

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1. Introduction

Prostate cancer is the most common non-cutaneous malignancy in males causing one in three tumor-associated deaths in Germany and the second most common cause of cancer-related death in the United States (1) (Krebs in Deutschland; www.krebsdaten.de). Androgen deprivation therapy is the cornerstone of the urooncological management of advanced hormone-sensitive prostate cancer (2). However, patients inevitably enter a castration resistant stage in which tumor progression occurs despite androgen deprivation. These metastatic castration resistant prostate cancer (mCRPC) patients have, until recently, received chemotherapy (docetaxel, mitoxantrone) as first line therapy (3,4). However, the introduction of novel and highly potent anti-androgens such as abiraterone or enzalutamide has led to a shift in the clinical practice and these agents are now also being used in chemo-naive mCRPC patients (5,6). Nevertheless, treatment of mCRPC remains a major challenge due to the heterogeneity of the disease and the inevitable development of therapy resistance. It is therefore of importance to better understand the biology of prostate cancer progression in order to develop improved therapeutic strategies.

Recent advances in comprehensive genomic profiling have identified four major signaling nodes that are most frequently altered in prostate cancer: i) the androgen receptor (AR) signaling pathway; ii) the PI3K pathway; iii) the Ras/Raf/MEK/ERK pathway; and iv) the retinoblastoma protein (pRB) signaling pathway (7). These pathways were altered at high frequencies in both primary tumors and metastatic samples in an exemplary study (7). The AR was identified as the most commonly mutated gene in prostate cancer with 56-100% of tumors examined containing mutated AR. Loss of PTEN, a negative regulator of the PI3K pathway, is a hallmark of prostate cancer and occurs in 42-100% of the tumors analyzed. The Ras/Raf/MEK/ERK pathway was altered in 43-90% of tumors and the pRB signaling pathway was altered in 34-74% of prostate cancers (7).

Importantly, extensive crosstalk and redundancy exists between these signaling pathways leading to the hypothesis that therapy resistance may readily develop when only one of the four pathways is targeted (7-9). This suggests that developing either novel therapeutic agents, or therapeutic regimes, that target a broader spectrum of pathway components may improve the clinical benefit of systemic therapy (Table I). In this review, we highlight the most commonly altered signaling pathways in advanced prostate cancer which need to be taken into account for the development of such new rational therapeutic strategies (Fig. 1).

2. Androgen receptor signaling

Androgen signaling is mediated through the androgen receptor (AR), a ligand-activated transcription factor that is the main therapeutic target of first-line therapies for advanced prostate cancer. The inactive form of the AR resides within the cytoplasm. Binding of androgens to the receptor leads to receptor activation and translocation into the nucleus, where the transcription of androgen-dependent genes is induced (10). Androgen-deprivation therapy (or chemical castration) is an effective first-line therapy for metastatic prostate cancer, but despite good initial responses, castrate-resistant prostate cancer commonly develops leading to tumors that are insensitive to androgen ablation (11).

Castration resistance can develop through several mechanisms, most commonly by gene amplification, activating mutations of the AR, or expression of AR splice variants which promotes maintenance of AR activity and signaling capabilities at castrate hormone levels (7,12-15). Interestingly, AR gene amplification has been detected in 28% of CRPCs after androgen ablation, but has not been observed in tumor samples without prior exposure to androgen deprivation (12). Receptor hypersensitivity to androgens can also occur in CRPC through increased receptor stability, enhanced nuclear localization and overexpression of nuclear co-activators (16,17). Furthermore, AR point mutations have been demonstrated to confer AR promiscuity leading to an activation by non-androgen ligands such as progesterone and estradiol (18,19). These observations suggest a selective pressure to maintain AR-mediated signaling in CRPC despite androgen deprivation conditions.

Based on this fact, CRPC remains to a certain extent sensitive to second-generation AR antagonists and hormone

Table I. Inhibitors of the AR, PI3K, Ras/Raf/MEK/ERK and RB pathway.

Agent	Pathway inhibition
Abiraterone	AR
Enzalutamide	AR
ARN 509	AR
CFG920	AR
Orteronel (TAK700)	AR
BKM120	PI3K
PX866	PI3K
BEZ235	PI3K/mTOR
GDC-0980	PI3K/mTOR
MK2206	AKT
GDC-0068	AKT
Everolimus	TORC1
Temsirolimus	TORC1
PD98059	MEK
U0126	MEK
PD184352 (CI-1040)	MEK
PD0325901	MEK
Selumetinib	MEK
RDEA119	MEK
SL327	MEK
Pimasertib (AS-703026)	MEK
BIX 02188	MEK
BIX 02189	MEK
AZD8330	MEK
TAK-733	MEK
Trametinib (GSK1120212)	MEK
AZD 5438	CDKs
SCH 727965	CDKs
Seliciclib	CDKs
Flavopiridol	CDKs
PD0332991	CDK4/6
LEE011	CDK4/6

Modified after (37,77).

synthesis blockers, which includes drugs that target AR signaling at the androgen production and conversion level and at the receptor level such as abiraterone or enzalutamid (20). However, it has been shown that inhibition of the AR receptor can lead to activation of other signaling pathways via crosstalk, such as the PI3K pathway. For example, AR blockade can lead to a reduced expression of the AR-responsive immunophilin FBPK5, a chaperone for the AKT phosphatase PHLPP, and ultimately an increased level of phosphorylated AKT, a downstream target of PI3K (11). Further, non-ligand mediated activation of AR signaling has been shown to be induced through crosstalk with other oncogenic pathways such as the mitogen-activated protein kinase (MAPK) pathway (21,22).

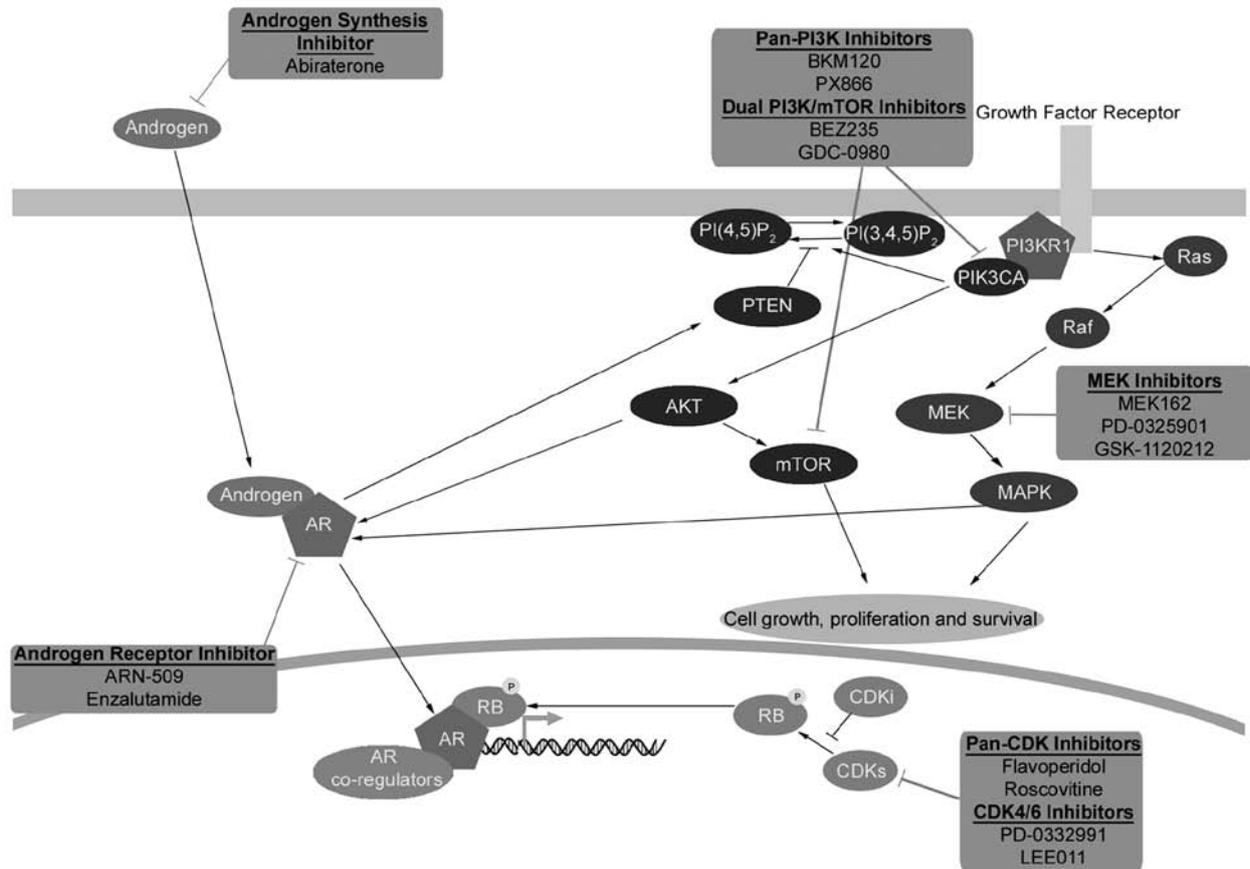


Figure 1. AR, PI3K, Ras/Raf/MEK/ERK and pRB pathway crosstalk and pharmacological inhibition. Recent advances in genomic profiling have identified four major signaling pathways that are most frequently altered in prostate cancer: i) the androgen receptor (AR) signaling pathway; ii) the PI3K pathway; iii) the Ras/Raf/MEK/ERK pathway; and iv) the retinoblastoma (pRB) signaling pathway. Extensive crosstalk and redundancy exists between these signaling pathways suggesting that devising a therapeutic regime that targets more than one of these pathways may provide a clinical advantage. Androgen signaling is mediated through AR, a ligand-activated transcription factor that is the main therapeutic target of first-line therapies for advanced prostate cancer. The inactive form of the AR resides within the cytoplasm, binding of androgens to the receptor leads to its nuclear translocation and transcription of androgen-dependent genes. Inhibition of the AR receptor can lead to activation of other signaling pathways such as the PI3K pathway through pathway crosstalk. The PI3K pathway is the second most frequently deregulated signaling pathway in prostate cancer behind only the AR signaling pathway. The PI3K pathway is normally activated following growth factor stimulation of receptor tyrosine kinases ultimately resulting in the activation of AKT, the central effector of many downstream signaling pathways regulating protein synthesis, cell cycle, cell death, cell growth and cell survival. AKT activation can be reversed by PTEN, which negatively regulates PI3K pathway activation. Another important target of the PI3K signaling pathway is the serine/threonine kinase mTOR (mammalian target of rapamycin). The Ras/Raf/MEK/ERK signaling pathway regulates fundamental cellular processes, including proliferation, differentiation and cell survival and is frequently activated in several cancer types, including prostate cancer. The PI3K and Ras/Raf/MEK/ERK pathways have been shown to interact extensively and frequent co-activation of these two pathways has been observed in prostate cancer. Ras/Raf/MEK/ERK signaling is stimulated by growth factor receptor activation of the small GTPase Ras, which in turn activates the protein kinase Raf leading to the activation of the dual specificity MAPK kinase (MEK1/MEK2), and finally phosphorylation of extracellular signal-regulated kinases (ERK1/ERK2). Ras/Raf/MEK/ERK pathway can sustain the transcription of androgen responsive genes, and therefore prostate cancer cell proliferation, even when the AR is inhibited. Retinoblastoma (RB1) is a tumor suppressor gene with somatic alterations in multiple cancers whose gene product (pRB) inhibits cell cycle progression. pRB activity is predominately controlled by cell cycle regulated cyclin-dependent kinase (CDK) activity. When CDK activity is inhibited, pRB remains in a hypophosphorylated and active state repressing E2F-regulated gene transcription and inhibiting cell cycle progression. Inactivation of pRB prevents cells from restraining cellular proliferation and leads to the aberrant expression of E2F-responsive genes. Dysregulation of E2F binding motif-containing genes is a hallmark of CRPC. Pharmacological inhibitors of the different signaling pathways are shown.

3. Pharmacological inhibition of androgen receptor signaling action

Abiraterone. Chemical castration leads to decreased production of testicular androgens but adrenal glands and even prostate cancer tissue can continue to produce androgens, which contributes to continued prostate cancer cell growth despite castrate level of androgens. Synthesized in the early 1990s, and approved for use in CRPC patients in 2011, abiraterone treatment promotes a global blockade of androgen production through irreversible inhibition of 17 α -hydroxylase/C17,20 lyase (CYP17), a key

enzyme involved in androgen synthesis. CYP17 is expressed in testicular, adrenal, and prostatic tumor tissues and inhibition of CYP17 results in a profound decrease of circulating androgens. Currently, abiraterone is used in mCRPC patients both prior to and post chemotherapy with or without mild symptoms to delay symptomatic disease progression (23,24). Side effects following abiraterone treatment include hypertension, decreased serum potassium, edema and increased adrenocorticotrophic hormone (ACTH) release. Other frequent adverse events include fatigue, fluid retention, hypokalemia, hypertension, cardiac disorders and liver enzyme increases (24,25).

Combination therapy with other androgen signaling pathway inhibitors, albeit with different mechanisms of action, for example enzalutamide and/or other therapeutic agents, are currently ongoing or in the planning phase (26).

Enzalutamide. Enzalutamide affects multiple steps in the AR signaling pathway including: i) competitive inhibition of androgen binding to the AR; ii) inhibition of nuclear translocation of the AR into the nucleus; iii) reduction of AR association with DNA; and iv) *in vitro* suppression of growth and induction of apoptosis in cell lines with AR gene amplifications (27).

Enzalutamide is well tolerated, most frequent side-effects reported were fatigue (33.6%), cardiac disorder (6.1%), myocardial infarction (0.3%), abnormalities like AST/ALT increase and bilirubin increase (1%). Seizures were rare and occurred in 0.6% of patients (28).

Enzalutamide is used in the post-chemotherapy setting and was found to improve progression-free survival (PFS), quality of life (QOL) and overall survival (OS) (29). Results of the PREVAIL phase III trial showed a significantly improved PFS and OS also in chemotherapy-naïve patients (6).

ARN-509. ARN-509 is a novel AR receptor antagonist that, unlike bicalutamide, does not show any agonist functions in the context of CRPC with AR overexpression. ARN-509 inhibits AR nuclear translocation and transcriptional activity and a phase I trial showed a favorable safety and side effect profile (26,30,31).

4. Phosphatidylinositol-3-kinase (PI3K) signaling

One of the key pathways essential to cell proliferation, survival, and metabolism is the phosphatidylinositol 3-kinase (PI3K) pathway (32). The PI3K pathway is the second most frequently deregulated signaling pathway in prostate cancer, behind only the AR signaling pathway (7). The PI3K pathway is normally activated following growth factor stimulation of receptor tyrosine kinases ultimately resulting in the conversion of membrane phosphatidylinositol-bis-phosphate (PI(3,4)P₂; PIP₂) to phosphatidylinositol-tri-phosphate (PI(3,4,5)P₃; PIP₃) by PI3K. This conversion of PIP₂ to PIP₃ by PI3K can be reversed by the tumor suppressor phosphatase and tensin homolog deleted on chromosome ten (PTEN) phosphatase, which functions to negatively regulate PI3K pathway activation. The formation of PIP₃ mediates the activation of AKT, which is the central effector of many downstream signaling pathways regulating protein synthesis, cell cycle, cell death, cell growth and cell survival (32).

Another important target of the PI3K signaling pathway is the serine/threonine kinase mTOR (mammalian target of rapamycin). mTOR occurs in two complexes, the TORC1 complex (mTOR bound to Raptor) and the mTORC2 complex (mTOR bound to Rictor). Both mTORC1 and mTORC2 are substrates of AKT: when activated, mTORC1 regulates protein translation, while mTORC2 can phosphorylate AKT and provide positive feedback to this branch of the signaling network (33).

Approximately 34-40% of primary and 74-100% of metastatic prostate cancers harbor alterations of components of the PI3K pathway, generally promoting aberrant pathway activation (7). These genetic aberrations frequently involve loss-of-function mutations, deletions, or epigenetic silencing

of PTEN, a negative regulator of PI3K, and/or activating mutations in PIK3CA, the catalytic p110 α kinase subunit (7,34-36).

Importantly, it has recently been shown that inhibition of the PI3K pathway in PTEN-deficient prostate cancer activates AR signaling by relieving feedback inhibition of the receptor tyrosine kinases HER2 and HER3. Conversely, inhibition of AR signaling was shown to activate AKT signaling through a reduced expression of the AKT phosphatase PHLPP (11). Reciprocal feedback regulation of the PI3K and AR pathways provides a compelling explanation for the poor efficacy of single-pathway inhibition therapy, for instance inhibition of the AR pathway alone, in PTEN-null cancers and the substantially improved antitumoral efficacy of combined PI3K/AR pathway inhibition. This pathway crosstalk may also be partially responsible for induction of castration resistance, which further underscores the importance of developing PI3K pathway targeting agents for the treatment of prostate cancer patients.

5. Pharmacological inhibition of PI3K signaling

The PI3K pathway is target-rich and a number of efforts have been made to exploit this fact for anticancer therapy. The PI3Ks are grouped into three classes of enzymes (I-III), leading to the development of both pan- and isoform-specific PI3K inhibitors as well as dual PI3K/TORC1/2 inhibitors (37). Several substances are currently under investigation as single agents or in combination with abiraterone (7-9).

The pan-PI3K inhibitor BKM120 (buparlisib) is currently under investigation in men with CRPC as single agent and in combination with abiraterone in two clinical trials (NCT01385293, NCT01634061). Treatment-related adverse events in BKM120-treated patients were most commonly fatigue, nausea, rash, hyperglycemia, diarrhea, anorexia and mood alterations (37-39). The latter underlines the need for close observation for psychiatric symptoms in patients treated with PI3K inhibitors that are able to cross the blood-brain barrier. Metabolic adverse events were most often reversible and not acutely toxic (40).

Isoform-specific PI3K inhibitors may be particularly relevant in patients in which PIK3CA is mutated (up to 16%) but redundancy between different isoform needs to be considered (7). Dual PI3K/mTOR inhibitor such as BEZ235 or GDC-0980 lead to a profound inhibition of PI3K signaling and have so far been well-tolerated. BEZ235 or GDC-0980 in combination with abiraterone is currently under investigation in a phase I/II clinical trials in men with mCRPC (NCT01717898). AKT inhibitors such as perifosine have so far not led to significant clinical responses (37,41). Again, combination therapies between antihormonal substances and PI3K inhibitors appear to be the most promising avenues for future drug development and results from ongoing phase II trials will be instrumental to corroborate this notion. Inhibitors of TORC1 such as rapamycin and its analogs everolimus or temsirolimus did not show significant antitumoral effects when used as single agents in men with CRPC (37,41).

6. Ras/Raf/MEK/ERK signaling

The Ras/Raf/MEK/ERK signaling pathway regulates fundamental cellular processes, including proliferation, differ-

entiation, and cell survival and is frequently activated in several cancer types including prostate cancer. Increased activation of this pathway correlates with a poor prognosis and tumor invasiveness (42-44). Ras/Raf/MEK/ERK signaling is stimulated by growth factor receptor activation of the small GTPase Ras, which in turn activates the protein kinase Raf leading to the activation of the dual specificity MAPK kinase (MEK1/MEK2), and finally phosphorylation of extracellular signal-regulated kinases (ERK1/ERK2) (45). The PI3K and Ras/Raf/MEK/ERK pathways have been shown to interact extensively and frequent co-activation of these two pathways has been observed in prostate cancer (46). Both pathways share common activation signals, such as receptor tyrosine kinase mediated activation, and also appear to provide compensatory signaling when one or the other is inhibited (47). Approximately 43% of primary and 90% of metastatic prostate tumors were found to harbor genetic alterations of the Ras/Raf/MEK/ERK pathway (7).

Although members of the Ras family are rarely mutated in general as well as in prostate cancer, the expression of important growth factor receptors promoting Ras activation, such as EGFR, FGFR and PDGFR, is frequently upregulated in prostate cancers (48). Further, ERK1/ERK2 activation is associated with increasing Gleason score and tumor stage (49). This is consistent with a functional role for the Ras/Raf/MEK/ERK signaling pathway as prostate cancer progresses to a more advanced, androgen-independent stage.

An activated Ras/Raf/MEK/ERK pathway could therefore provide a selective advantage to tumor cells under androgen-deprivation pressure. This notion is corroborated by the finding that the Ras/Raf/MEK/ERK pathway can sustain the transcription of androgen-responsive genes, and therefore prostate cancer cell proliferation, even when the AR is inhibited (49,50).

7. Pharmacological inhibition of the Ras/Raf/MEK/ERK pathway

Efforts to target Ras directly have not been successful in the clinic to date, but recent clinical trials with Raf and MEK inhibitors have suggested that targeting these downstream Ras effectors could be promising.

Single agent therapy with MEK inhibitor alone showed a compensatory upregulation of the PI3K pathway as well as several others including NF- κ B and hedgehog as an expression of cellular pro-survival mechanisms (51). Dual inhibition of both MEK and PI3K/mTOR signaling increases apoptosis in cell lines and dual inhibition of AKT/mTOR and ERK has been demonstrated to lead to effective growth inhibition in mouse models of prostate cancer (46,52). Furthermore, ERK inhibition was found to enhance docetaxel-induced cytotoxicity in androgen-independent prostate cancer cells (63).

Phase III trials have been performed with GSK-1120212 (trametinib), which is an FDA-approved MEK1/2 inhibitor, for metastatic melanoma showing a favorable safety profile. Main adverse events were acneiform skin alterations, diarrhea, peripheral edema, hypertension and transient mild cardiac dysfunction. Most toxicities did not require drug discontinuation (54-57). Trials are going to be initiated in patients with different tumor entities (58). Other MEK inhibitors such as MEK162 (binimetinib) and PD-0325901 are likewise in phase I/II clinical trials.

8. Retinoblastoma protein (pRB) pathway

The retinoblastoma gene (RB1) is a tumor suppressor gene with somatic alterations in multiple cancers whose protein product (pRB) inhibits cell cycle progression (59). In prostate cancer, pRB inactivation through genomic deletion of RB1 has been reported in approximately 20-60% of tumors and is associated with transition to CRPC and poor clinical outcome (60,61). pRB activity is predominately controlled by cell cycle regulated cyclin-dependent kinase (CDK) activity (58). When CDK activity is inhibited, pRB remains in a hypophosphorylated and active state repressing E2F-mediated gene transcription thereby inhibiting cell cycle progression (58). Inactivation of pRB prevents cells from restraining cellular proliferation and leads to an aberrant expression of E2F-responsive genes (62). Overexpression of cyclins also contributes to the deregulation of pRB function in prostate cancer (7,63,64). Remarkably, CRPC tissue has been found to show an altered repertoire of AR binding sites that were enriched for E2F motifs, which can lead to cyclin/CDK hyperactivity, pRB inactivation and uncontrolled proliferation (61).

Although it was demonstrated that pRB-deficient tumors respond poorly to hormone therapy, increasing evidence suggests that tumors with reduced pRB expression exhibit a more beneficial initial response to chemotherapy (65). Thus, RB1/pRB status could be a predictive marker of response to chemotherapy, with a potential to influence clinical decision-making, and a potential biomarker of transition to castration resistance. In addition, treatment with anti-androgens can lead to more aggressive tumors with neuroendocrine differentiation which is often associated with a loss of pRB protein expression (66).

9. Pharmacological inhibition of pRB signaling

Numerous studies have explored CDK inhibitors to target tumor cells with inactivated pRB and E2F-induced cyclin/CDK hyperactivity. The pan-CDK (CDK1, CDK2, CDK4/6) inhibitor flavoperidol was found to enhance apoptosis in androgen-independent PC-3 prostate cancer cells (67). An increase in apoptosis and a decrease in angiogenesis was detected when combined with docetaxel in a mouse model of prostate cancer (68,69). In addition, it has been shown that CDK1 can phosphorylate the AR at serine 5115 which was associated with unfavorable patient outcome. The CDK inhibitor roscovitine was found to reduce AR phosphorylation, which could be exploited clinically (70). A selective CDK4/6 inhibitor, PD-0332991 (palbociclib), showed a growth suppressive effect in prostate cancer xenografts. Phase I studies in colorectal cancer and multiple myeloma are ongoing (71). The CDK4/6 inhibitor LEE011 is currently under investigation in several phase I and II trials for advanced solid tumor, breast cancer, lymphoma and melanoma (72). Possible side effects known from clinical trials with roscovitine are nausea, vomiting, transient elevations in serum creatinine and liver enzymes.

10. Conclusions and outlook

Due to extensive crosstalk between signaling pathways such as the AR and PI3K pathways, PI3K and Ras/Raf/MEK/ERK pathway and pRB and AR signaling, single-agent therapy is

likely to result in an activation of mechanisms that thwart the antitumoral response, lead to drug resistance and ultimately treatment failure. Future drug and clinical trial development therefore needs to reflect the extensive feedback mechanisms that exist between the four most frequently altered signaling nodes in prostate cancer. However, there are a number of questions and concerns that need to be addressed. First and foremost, it will be important to determine the safety, tolerability and the actual clinical effectiveness of treatment regimen that encompass two or more pathway-targeting drugs. Given that unexpected and paradoxical survival pathway activation in response to targeted agents has been reported in other malignancies (73-76), it is possible that, depending on the genetic background and intratumoral heterogeneity, combination therapies that have been found to be effective *in vitro* and in preclinical models may not show the same efficacy when used in cancer patients.

Second, toxicity profiles for combination targeted therapies need to be carefully analyzed and weighed against the clinical benefit. Whether and to what extent a dose reduction of individual compounds is permissible without impeding the oncological effectiveness as it has been shown for 'classical' chemotherapeutic agents remains to be determined. Nonetheless, it is worth remembering that combination drug therapies were a major breakthrough in the chemotherapeutic treatment of several malignancies including Hodgkin's lymphoma and several others. So far, toxicity profiles of newer generation pathway inhibitors appear to be manageable but unexpected pharmacological interactions and resistance mechanisms always need to be considered.

Third, regulatory limitations are a major concern. Effective drugs may not be manufactured by the same pharmaceutical company and may lack approval in certain geographic regions. How to deal with this problem and whether multi-pathway targeting drugs are an attainable solution remains to be determined.

Nonetheless, from a purely biological point of view, novel treatment strategies for prostate cancer need to reflect the highly disorganized and profoundly interconnected signaling landscape that has emerged from genome-wide analyses.

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