Managing Metastatic Prostate Cancer in Your Urological Oncology Practice

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Preface

Management of castration-resistant prostate cancer (CRPC) has undergone a transformative change since the turn of the twenty-first century. Prostate cancer that progresses to castration resistance remains incurable and none of the treatment modalities tried and tested up to the twentieth century showed an unequivocal improvement in overall survival. However, within a span of a decade six drugs from different classes including chemotherapeutics, second generation antiandrogens, immunotherapeutics, and radio-isotopes were approved for human use based on data from large Phase III trials demonstrating improvement in overall survival in patients with CRPC. The improvements in survival are a modest 2–4 months in general, which highlight the opportunities and challenges that lie ahead for the field.

The concept of this textbook is to integrate basic, translational, and clinical science data to provide the reader with the understanding on how we got here, what direction we are heading, and where we need to be. Medical care for patients with prostate cancer is often provided by a variety of subspecialists through the continuum of the disease from apparently localized and often indolent disease through invariably fatal CRPC. The wide variability in the course of disease calls for in-depth understanding of the disease states and therapeutic options among all the medical subspecialists caring for men with prostate cancer. The book is designed to be of interest to various subspecialists involved in the care and to provide necessary, up-to-date information.

The lead authors in this book are leaders in the field with several original contributions to their credit. I am most grateful for their expertise and contributions, and the quality will be readily evident to the readers. While most readers may be familiar with androgen signaling in prostate cancer, there is a large body of basic science evidence supporting role of nonandrogen signaling in prostate cancer. A dedicated and detailed chapter is included on nonandrogen signaling in prostate cancer to familiarize the reader and provide a resource to follow this rapidly evolving area of prostate cancer research, which is likely to provide for future novel management strategies.

For men and families dealing with prostate cancer, much has been done but much more needs to be done. Undoubtedly, rapid advances will happen in the future in our understanding of CRPC and translation of newfound knowledge to management of patients. A common theme will remain throughout the dynamic process; the more we learn, it will be clearer what remains to be learnt.

K.C. Balaji

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This book is dedicated to men and their families who fought and continue to fight prostate cancer with courage and compassion. Many thanks to individual researchers, collaborators, industry, funding agencies, patients, families, and others for their invaluable efforts over decades unraveling the mysteries of prostate cancer, which produced the knowledge in this book. A special thanks to my parents and brothers for their support through the years, and my wife Shoba, children Navin and Nandita for parting with their family time to make this book happen. I deeply appreciate the contributions of my teachers, mentors, colleagues, family, and friends over the years to my training and development, whose insights helped me immensely with designing the content for this book. Most importantly, I remain grateful to the authors in this book for their expertise and contributions.

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Introduction

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Prostate cancer in the most frequently diagnosed non-cutaneous cancer in western countries. While most men with prostate cancer are diagnosed with apparently localized and often indolent disease, a vast majority of men dying from prostate cancer either present with metastatic disease or high risks disease such as Gleason grade >7 or non-organ confined disease [1]. Unlike some of the other human metastatic cancers that are rapidly fatal, metastatic prostate cancer although incurable, may have a prolonged course and thereby necessitates management strategies of a chronic disease. The median survival for men with metastatic prostate cancer is about 5 years [1]. Androgen deprivation therapy (ADT) has been the sheet anchor for men diagnosed or suspected to have metastatic prostate cancer for decades. While ADT has proven benefit in symptomatic improvement in men with metastatic prostate cancer, the evidence supporting improvement in survival with primary ADT alone for localized or metastatic prostate cancer is much less convincing. Prolonged ADT is fraught with significant and severe side effects of which progression to castration resistance in about 3 years seems inevitable [2].

The turn of 21st century saw a glut of treatment options in management of men with advanced prostate cancer [3]. Unlike prior efforts at identifying effective chemotherapeutic agents, Docetaxel was proven to improve survival in men with CRPC by two large randomized contemporaneously performed independent Phase III clinical trials [4, 5]. While Docetaxel improved survival in men with metastatic prostate cancer, resistance to the drug and progression of disease eventually ensued creating a post-chemotherapy space. An improvement in survival was

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demonstrated by another large Phase III randomized in men progressing on Docetaxel chemotherapy using second line chemotherapy with Cabazitaxel belonging to the same family of taxanes but with distinct mechanisms of actions [6].

During this period of historic clinical advances, basic research demonstrated that while serum testosterone remains low in men on ADT, sufficient levels of androgens were available in prostate cancer tissue driving disease progression. Strategies to combat tissue androgens led to development and initial approval of Abiraterone, a Cyp17A inhibitor that decreases androgen synthesis, and Enzalutamide, a second-generation androgen receptor antagonist in post-chemotherapy space. These drugs were later proven to improve survival in men who have not undergone Docetaxel chemotherapy as well and therefore commonly used as first line therapy in men progressing to castration resistance [7–10].

While second generation anti-androgens were independently studied in pre- and post-chemotherapy spaces, two other drugs with different mechanisms of actions were studied in patients with or without prior Docetaxel chemotherapy. Sipuleucel-T was the first cancer vaccine approved for use in humans based on improvement in overall survival in men with CRPC demonstrated by 2 large Phase II and a Phase III clinical trial in patients with minimally symptomatic disease [11]. Radium-223, an alpha-emitting radioisotope was approved treatment of men with CRPC and symptomatic bone metastasis following demonstrable improvement in overall survival in a large Phase III trial [12]. A total of 6 novel therapeutic agents were approved within a decade for men with metastatic CRPC, which led to additional questions such as use of agents earlier in the course of disease and sequencing.

Three large Phase III studies using Docetaxel concurrently with ADT were done in men with androgen sensitive metastatic prostate cancer, of which 2 studies showed dramatic improvement in survival of over a year in men receiving Docetaxel with ADT compared to ADT alone in men with large volume disease [13]. Because the novel agents have shown improvement in overall survival in men who have received prior chemotherapy, the real question is sequencing of available agents. The cross-resistance between second-generation anti-androgens Abiraterone and Enzalutamide is apparent [14]. The choice of agents is likely to depend on patient symptoms, need for prescribing steroids with Abiraterone, side effect profile, costs and availability.

A common theme among all studies done in men with CRPC is improvement in overall survival ranging 2–4 months. While sequencing of medications may provide cumulative survival benefit, the fundamental challenge of innate or acquired resistance to novel agents and disease progression perhaps due to heterogeneity of prostate cancer remains to be addressed. In each of the class of novel agents approved newer agents or dosing options are being studied. While androgen signaling is well studied and successfully targeted to date, emerging data suggest major role for non-androgen signaling pathways in prostate cancer that could lead to additional diagnostic and therapeutic strategies. This text book details various studies that have led to the advances in management of men with CRPC, highlights

ongoing work in the field, future directions and could be used a reliable resource for providers and researchers involved in addressing the needs of men with advanced prostate cancer.

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Pathophysiology of Castration-Resistant Prostate Cancer

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Justin C. Penticuff and Natasha Kyprianou

Abbreviations

CRPC	Castration resistant prostate cancer
PSA	Prostate specific antigen
ADT	Androgen deprivation therapy
DHT	Dihydrotestosterone
AR	Androgen receptor
DHEA	Dehydroepiandrosterone
AD	Androstenedione
HSP	Heat shock protein
IAP	Inhibitors of apoptosis proteins
PTEN	Phosphatase and tensin homolog
BTG1	B-Cell translocation gene 1
BCL2	B-Cell CLL/Lymphoma 2
IGF-1	Insulin like growth factor 1
KGF	Keratinocyte growth factor
EGF1	Epidermal growth factor 1
TIF2	Transcriptional intermediary factor 2
RTK	Receptor tyrosine kinase
MAPK	Mitogen activated protein kinase
STAT	Signal transducer and activator of transcription
AF-1, 2	Activating function
NTD	N-terminal domain

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DBD	DNA binding domain
LBD	Ligand binding domain
NLS	Nuclear localization signal
ARV	Androgen receptor variant
ASO	Antisense oligonucleotide
LHRH	Lutenizing hormone-releasing hormone
MAB	Maximum androgen blockade
CVD	Cardiovascular disease
IAD	Intermittent androgen deprivation
FDA	Food and Drug Administration
SWOG	Southwest Oncology Group
FOXO1	Forkhead box O1
HSET	Human kinesin-14
MCAK	Mitotic centromere associated kinesin
Src	Proto-oncogene protein tyrosine kinase Src

Background

Clinical Development of Castration Resistant Prostate Cancer (CRPC)

In the United States prostate cancer is the most commonly diagnosed malignancy, and remains the second most common cause of cancer related mortality second only to lung cancer. In 2014, there were an estimated 233,000 new cases of prostate cancer diagnosed and 29,480 deaths due to prostate cancer [1]. The incidence rate of prostate cancer declined −2.1 % from 2000–2010, likely reflecting improved prevention and variable utilization of prostate specific antigen (PSA) screening methods nationwide, which remains controversial [1]. The historic studies by Huggins and Hodges in 1941 [2], established the role of androgens as the primary driving force in prostate tumor growth, with androgen removal resulting in dramatic suppression of tumor growth. Androgen-deprivation therapy (ADT) stems from the recognition that circulating androgen levels (primarily testosterone (T) and dihydrotestosterone (DHT)) are responsible for the embryonic development, differentiation, maturation, and consequently, malignant growth of the prostate [3]. Fortunately, improved screening and detection has resulted in roughly 80 % of new cases being diagnosed as early-localized disease. A large number of patients (33–40 %) diagnosed with early-localized disease treated with radical prostatectomy develop recurrence of disease or progress to metastases [4]. ADT remains the gold standard for patients with metastatic, advanced disease. Androgen deprivation therapy has been achieved via multiple methods including surgical orchiectomy, targeting the hypothalamic-pituitary axis via GnRH agonists and antagonists, blocking steroid production by enzymatic inhibition, and via antiandrogens that

inhibit binding to the androgen receptor (AR). Initial response to ADT is often dramatic, with 80–90 % of patients achieving rapid decline in serum PSA, and reduction of serum testosterone to 'castrate' levels (<50 ng/mL) [5, 6]. After an an average remission time of 2–3 years, nearly all patients progress to castration resistant prostate cancer (CRPC), defined by rising serum PSA reflecting activation of AR transcriptional activity, or appearance of metastases via imaging [5, 7]. Approximately 90 % of patients with CRPC will develop bone metastases resulting in severe pain, pathologic fractures and/or bone marrow failure [7]. Advanced CRPC is ultimately lethal, with median survival of 18–24 months from initiation [3].

Mechanisms Driving CRPC Progression to Metastasis

Identifying the hormonal and physiological mechanisms driving the transition to androgen-independent state has been a critical determinant to drug development and therapeutic outcomes during cancer progression to metastasis. While testosterone is the dominant circulating androgen, DHT is the primary intracellular androgen in the prostate gland, serving as the ligand for AR. DHT is produced by intraprostatic 5-α reductase enzyme from testosterone. About 10 % of circulating androgens are derived from the adrenal cortex [dehydroepiandrosterone (DHEA), androstenedione (AD)] and are also converted to testosterone within prostatic cells [8]. AR is present in the cytoplasm of secretory epithelial cells as well as surrounding stromal cells. In the absence of ligand binding, AR is bound to various chaperone molecules including heat shock proteins (HSP). Upon binding androgens, conformational changes occur within AR, allowing for dissociation from chaperone molecules and translocation of the AR-androgen complex to the nucleus where specific DNA sequences (androgen responsive elements) are bound, resulting in production of proteins that enable prostate epithelial cells and stromal cells to proliferate. Importantly, absence of androgen initiates apoptotic signaling within the stromal and epithelial compartments. The progression of tumor growth and development of metastases despite reduction of serum androgens to 'castrate' levels is dependent upon the utilization of adaptive cell-survival pathways [9–11]. Mechanisms contributing to CRPC progression include intratumoral production of androgens via increased expression of steroidogenic enzymes, apoptosis evasion, altered AR transcriptional coregulator expression, AR posttranslational modification (phosphorylation), ligand-independent pathways activating AR, amplification, and selection of genetically modified AR with constitutive active AR splice variants [3, 5, 6, 12].

Intratumoral Steroidogenesis

How can intratumoral androgen levels remain high after ADT? In the absence of gonadal androgen synthesis following ADT, the adrenal precursor DHEA is converted to androstenedione by 3β-hydroxysteroid dehydrogenase within the prostate

[13]. Androstenedione is then converted to DHT via 5-α reductase (SRD5A1, SRD5A2). Inhibiting adrenal steroid production by administration of abiraterone acetate remains a cornerstone of CRPC treatment regimens. Abiraterone acetate inhibits CYP17A1, breaking the pathway to DHEA, T, and estradiol in both the testes and adrenal glands [6]. Recent work however, demonstrates that DHT synthesis is dominated by an alternative enzymatic pathway in CRPC. In this 'back-door' pathway, progesterone is converted to androstenedione in a series of enzymatic steps that does not require CYP17A1, and is then converted to 5α-androstenedione after 5α-reduction by SRD5A1, which is then converted to DHT, thus bypassing the action of steroid synthesis inhibitors [13, 14]. Compelling data suggests upregulation of a heterogeneous group of enzymes responsible for steroid synthesis from both adrenal precursors as well as cholesterol precursor (CYP17A1, HSD3B1, FASN, HSD17B3, CYP19A1, UGT2B17, etc.) in CRPC metastases [3, 15, 16]. Cholesterol is the primary substrate for the synthesis of steroid hormones, and its altered production is implicated in CRPC development. Increased cholesterol influx via SRD-1 and LDL, increased synthesis via upregulated HMG-CoA reductase, and increased formation of free cholesterol from intracellular cholesterol ester stores contribute to intratumoral androgen synthesis in CRPC [17]. Thus impaired cholesterol production via the use of HMG-CoA reductase inhibitors (statins) reduces intratumoral androgens in CRPC. The wealth of epidemiological evidence on the ability of statins to confer a reduced risk of developing advanced prostate cancer [18], directs an ongoing randomized trial on the use simvastatin in CRPC patients [19].

Dysregulation of Apoptotic Pathway

Programmed cell death is an intricately orchestrated cellular process that commences with activation of either the extrinsic (cell surface death receptor activation) pathway or the intrinsic (mitochondrial; release of cytochrome c) pathway with the end result being organized degradation of cellular organelles and machinery by proteolytic enzymes. The extrinsic pathway and intrinsic pathway converge at activation of the 'effector' caspase-3 [20]. The commitment to apoptotic pathways depends upon the Bcl2 protein family members, that functionally interact with inhibitors of apoptosis proteins (IAPs) to determine apoptotic outcomes and cell survival. Within the Bcl2 family resides both pro-apoptotic proteins (Bim, Bad, Bak, Bax, etc.) as well as anti-apoptotic proteins (Bcl-2, Bcl-cL, Bcl-xL, Mcl-1, etc.) whose balance is essential for both embryogenesis and normal tissue growth and maintenance [20]. Disruption of this molecular balance in favor of expression of anti-apoptotic proteins leads to apoptosis evasion, aberrant tumorigenesis, loss of androgenic control, therapeutic resistance, increased metastasis and shortened survival [20, 21]. Expression of anti-apoptotic Bcl-xL correlates with prostate cancer progression progression [21], and can be targeted by ADT, indicating dependence on AR signaling [21]. During progression to metastatic CRPC, Bcl-xL levels are significantly higher than primary tumors, correlating with activated ligand-independent AR signaling [21]. This outlaw pathway of AR activation

navigated by apoptosis regulators can be overcome by inhibition of Bcl2 sensitizing CRPC tumors to chemotherapy [22]. Loss of tumor suppressors p53 and PTEN characterizes poorly differentiated tumors with treatment failure outcomes. Moreover loss of p53 contributes to apoptosis resistance by loss of its regulatory activation of Bax [23]. Functional loss of PTEN results in unregulated and constitutive activation of the PI3K/Akt pathway, which contributes to cell growth and survival [23]. The role of micro-RNAs in altering the molecular landscape of CRPC in the context of apoptosis regulation has recently been recognized with potential therapeutic value. Expression of miR-19a is associated with emergence to CRPC via inhibition of the BTG1 tumor suppressor gene which regulates Bcl2 expression [24]. Inhibition of miR-19a significantly induces apoptosis in CPRC cells, highlighting the functional relationship between miRNAs and apoptosis control in CRPC [24].

AR Bypass Pathways Navigated by Co-Regulators

The development of CRPC is due in large part to continued AR signaling made possible by aberrant and unregulated signaling of mutated or alternatively spliced AR that is no longer dependent upon androgen binding to ensure its activation. However, many other native cellular signaling cascades are altered in advanced disease that contribute to enhanced activity of AR and development of CRPC under conditions of castrate androgen levels. Insulin-like growth factor-1 (IGF1), keratinocyte growth factor (KGF) and epidermal growth factor (EGF) have been shown to contribute to the activation of AR in absence of androgen. Interestingly, when subjected to anti-androgen therapy (AR direct blockade) IGF1, KGF, and EGF1 were no longer able to induce AR activation and transcription of target genes, implying a direct link between these proteins with AR [25]. IGF1 induces AR signaling by upregulating expression of various AR co-activators including TIF2 [11]. Compelling evidence suggests that overexpression of p160 co-regulator proteins (SRC1, SRC2, TIF2, etc.) following ADT, can impact both androgen dependent and androgen independent effects on AR activation in CRPC under androgen-depleted conditions 14. As these growth factors are ligands for receptor tyrosine kinases (RTKs), the cross talk between these signaling pathways in CRPC is prominent. Receptors for IGF1 and EGF (both RTKs) are well known to affect downstream signaling activation of various cell growth and survival pathways including AKT, MAPK, and STAT pathways, all of which are activated in CRPC [11]. One the major RTKs that has been heavily involved in several human malignancies is HER-2/neu and its signaling.

Overexpression of HER-2/neu has been shown to increase transcription of PSA, even under conditions of androgen depletion, supporting a dynamic cross-talk between RTK and AR pathways in the absence of ligand [26]. The Wnt/β-Catenin signaling pathway is implicated in a variety of cancers, by mechanistically contributing to cell self-renewal [27]. In the presence of androgens, stabilized β-Catenin co-localizes to the nucleus with AR and promotes its transcriptional activity, acting as an AR coactivator [27]. At castrate androgen levels mimicking

CRPC, AR signaling engages the Wnt/β-Catenin pathway, and conversely, stabilized β-Catenin can in turn promote AR transcriptional activity, indicating the importance of the Wnt/AR crosstalk in CRPC development [27].

The cytokines interleukin-6 and -8 (IL-6) and (IL-8), under the regulation of NF-κB signaling pathway, can also enhance the expression of AR target genes in a dose dependent, paracrine manner in androgen depleted conditions [11, 28]. Androgen independent MDA PCa 2b cells are growth inhibited in response to antiandrogens in the presence of IL-6 and IL-8 [28], supporting a dynamic exchange between AR and these cytokines. Additional signaling networks mediated by RAS/MAPK, TGF-β, FGF, c-MET, can causally interact with AR towards the emergence of CRPC, and their functional involvement and targeting consequences are being pursued.

Discussion

The Identity of the Androgen Receptor

The complexity of AR and its varied mechanisms and alterations exert an important role in embryogenesis, pubertal development, the physiologic dysregulation accompanying male pattern baldness and prostatic hyperplasia, and the development of prostate cancer. The androgen receptor is grouped into the steroid and nuclear receptor superfamily, which also consists of glucocorticoid, mineralocorticoid, estrogen, and progesterone receptors. The AR is transcribed from the AR gene, which is located on Xq11-12 and contains eight exons that encode a protein of roughly 919 amino acids, with its varying length in individuals afforded by variable length polyglutamine and polyglycine repeat sequences [14]. Genomic organization of AR has been highly conserved throughout mammalian evolution, and is characterized by presence of four functional motifs: an N-terminal domain (NTD) that regulates transcription via activation function-1 (AF1) units, a central DNA binding domain (DBD) comprised of two zinc fingers, a C-terminal ligand binding domain (LBD) that contributes to transcription regulation via activation function-2 (AF2) units, and a small hinge region between the DBD and LBD that contributes to nuclear localization and degradation [29–31]. Once translated, unliganded AR resides in the cytoplasm bound to chaperone proteins, most commonly heat shock protein 90 (Hsp90), and will inevitably undergo degradation by proteasomes in the absence of ligand (T or DHT) [30]. Once ligand binds AR LBD, conformational shifting of various helices releases AR from Hsp90 binding and results in the stabilization of bound ligand as well as the generation of a hydrophobic cleft motif responsible for subsequent binding of co-regulator proteins, of which more than 150 have been identified [14, 30]. The nuclear localization signal present in the hinge region (NLS) is revealed during this conformational shift, resulting in translocation of the dimerized AR-ligand complex to the nucleus (via ATP dependent dynein motor proteins) where binding of DNA at androgen

response elements (ARE) results in formation a multi-protein complex after recruitment of multiple co-activators and co-repressor proteins that serve to regulate target gene transcription [14, 29, 32]. Many of the co-regulators are enzymes serving to remodel tightly bound chromatin structures to enable efficient transcription of DNA [29].

In the adult prostate, AR is located in luminal cells of prostate glandular tissue and surrounding stromal cells, and in a minority of basal epithelial cells and intermediate cell types of the epithelial compartment. Prostate glandular development and proliferation is dependent upon the paracrine effects of stromal cells. Once bound by circulating androgen, stromal AR induces the production of soluble paracrine factors termed 'andromedins' which diffuse across the epithelial basement membrane and mediate epithelial compartment proliferation [8, 33]. Interestingly, AR is growth stimulatory in luminal cells while it is inhibitory in basal cells highlighting its important regulation of normal prostate growth [34].

The AR Addiction: "Friend or Foe" in CRPC Treatment

AR Amplification

Amplification of the AR gene with resultant increased expression of AR target genes is a primary mechanism driving uncontrolled prostate tumor growth under conditions of androgen depletion. This state of "AR addiction" increases the probability of binding ligand in an androgen-depleted environment. In approximately one third of CRPCs treated with androgen deprivation therapy, amplification of AR is present [35]. In CRPC not treated with ADT, AR amplification is not found, pointing to clonal selection of those cells capable of AR amplification under conditions of very low androgen to retain AR signaling [8, 35, 36]. Amplification of AR is achieved by X chromosome rearrangements and polysomy in roughly 60 % of CRPC initially [36, 37]. Amplification of AR contributes to dramatically increased sensitivity of AR to very low levels of androgen, especially DHT. The concentration of DHT required for growth stimulation in CRPC tissues has been shown to be four orders of magnitude lower than that of primary tumors naïve to hormonal ablation [38]. AR amplification coincides with increased AR stability, increased AR nuclear localization and amplification in recurrent tumors does not appear to affect survival [36, 38]. Intriguingly, Chen et al. revealed that in the setting of AR amplification, administration of the antiandrogen bicalutamide, as well as other androgen receptor antagonists, led to increased AR target gene expression suggesting that in the setting of elevated AR, antagonists are converted to weak agonists [39].

AR Mutations and Promiscuity

Mutations of the androgen receptor are quite rare in early stage, untreated prostate cancer but are very common in CRPC, occurring in roughly 10–30 % of cases, suggesting clonal selection as an adaptive response to androgen ablation and

antiandrogen therapy [40, 41]. The highest frequency of AR mutations in CRPC occurs in patients treated with antiandrogens such as flutamide (~30 % of cases vs. ~5 % treated with castration alone) [41]. More than 660 mutations of AR have been reported, most of which are single base substitutions that have varying effects (gain of function, loss of function, null) of AR function depending on their location [42]. Roughly 49 % occur in the LBD, 40 % in the NTD, 7 % in the DBD, 2 % in the hinge region, and very rarely in untranslated regions [43]. The commonly occurring AR mutations in CRPC affect the ligand binding, reducing specificity and increasing promiscuity of binding to non-androgen ligands. The first AR point mutation identified in prostate cancer was identified in the LNCaP cell line, and occurs at codon 877, resulting in substitution of alanine for threonine (T877A). This mutation remains the most frequently occurring point mutation in CRPC AR, and results in an altered binding pocket that facilitates binding to various other hormones including estrogen, progesterone, various corticosteroids, and a select few antiandrogens (cyproterone and hydroxyflutamide) which confers a survival advantage within an androgen scarce environment [41, 43]. In pre-clinical models of CRPC, treatment with enzalutamide, an AR inhibitor, has been shown to a AR mutation F876L, resulting in the conversion of enzalutamide into an AR agonist (antagonist-to-agonist switch) [44]. Treatment resistance was demonstrated both in vitro and in vivo [44]. Several other mutations within the LBD result in increased AR transcriptional activity in the presence of various steroid hormones and include H874Y, L701H, V715M, V730M, [40, 41, 43, 45] etc. Importantly, point mutations within the NTD (G142V, M523V, G524D, and M537V) have been shown to induce development of constitutively active, mutant ARs with ligand independent activation, most likely due to increased interaction with p160 family of coregulator proteins [46].

AR Splice Variants

Androgen deprivation therapy for CRPC relies on the presence of a full-length AR with an intact LBD. The novel antiandrogen therapy approved for advanced disease, enzalutamide, exerts its effect by binding to and blocking the C-terminal LBD of intact AR, silencing transcriptional activity. The explosive evidence accumulating during the last few years on elucidation of the AR splice variants and the characterization of their clinical relevance in CRPC progression to advanced disease, has enhanced our understanding of the adaptive responses of prostate tumor cells to antiandrogen therapies. AR splice variants (AR-Vs) are the result of insertion of cryptic exons downstream of sequences encoding DBD, or deletions within the LBD that lead to disruptions in the AR open reading frame, and production of truncated AR lacking LBD, rendering them impervious to commonly utilized antiandrogen agents including enzalutamide [43, 47]. These ARVs are constitutively active mutants capable of regulating target gene expression in the absence of full length AR or androgen [48]. The exact mechanisms that lead to variable gene splicing of AR are poorly understood [41]. A loss of LBD, which normally functions as a repressor for the rest of the receptor, results in exposed and

functioning transactivation domains enabling initiation of gene transcription in the absence of ligand [41]. Among the family of newly identified AR-Vs, AR-V7 and ARv567 are the two most commonly occurring and clinically relevant. Both of these variants are induced by castration, and in men with CRPC bone metastases, their presence is a marker of particularly poor prognosis [48]. In a landmark study, Antonarakis et al. have recently demonstrated that prostate cancer patients harboring AR-V7 variants in circulating tumor cells showed no appreciable benefits from enzalutamide or abiraterone therapy, highlighting the AR-V7 as an important predictor of CRPC resistance [49. Multiple strategies to target the various other domains (NTD, DBD, etc.) with novel agents are currently being investigated [37]. Targeting exon 1 of AR with antisense oligonucleotide approaches suppresses both the full length AR and AR-Vs in enzalutamide resistant pre-clinical CRPC models [50].

Therapeutic Challenges in CRPC

Androgen Deprivation Therapy

In men with locally advanced and symptomatic metastatic prostate cancer, ADT remains the treatment of choice. In advanced prostate cancer, ADT has been demonstrated to delay progression of disease by reducing extraskeletal metastases, spinal cord compression, and ureteral obstruction, although it has not been shown to significantly increase overall survival [51]. ADT has been achieved in the past with surgical orchiectomy, but today, is achieved with equally efficacious administration of luteinizing hormone-releasing hormone (LHRH) agonists, LHRH antagonists, and anti-androgens. ADT was initially used as a primary treatment in symptomatic metastatic disease, or localized disease in patients in which radiation therapy or surgery is contraindicated, as adjunct treatment in high-risk disease treated with radiation therapy, and as a salvage therapy following biochemical failure after surgery or radiotherapy [52]. The 1967 Veterans Administration Cooperative Research Group study, a randomized controlled trial with 2052 men with clinically advanced prostate cancer receiving either ADT, revealed no significant difference by ADT in Five-year overall survival [53]. Co-administration of LHRH agents with antiandrogens provides a modest improvement in overall survival, but significantly impairs quality of life [54]. ADT increases CVD incidence and mortality, increases bone loss and fracture risk, while impairing erectile function, and memory function [55]. ADT has no significant impact in the treatment of localized prostate cancer [56]. In men with locally advanced and metastatic cancer, whose tumors associated with rapid PSA doubling times and an initial PSA >50 ng/mL, there is a benefit from early ADT by delaying progressive disease [57]. However, overall survival is not affected by the timing of ADT either in men with locally advanced asymptomatic disease, or in men with biochemical recurrence (rising PSA) after radical prostatectomy [54]. Importantly, intermittent androgen deprivation (IAD) therapy with periods allowing for hormonal recovery, versus continuous androgen deprivation (CAD), is associated with significant improvements in quality of life while

maintaining similar survival (8.8 years vs. 9.1 for IAD and CAD respectively) [52, 54]. ADT provides rapid biochemical response with dramatic decline in PSA in roughly 90 % of patients, and can offer remission from clinically symptomatic disease for 2–3 years [10]. And then the inevitable progression to resistant disease despite castrate androgen levels resulting in CRPC.

Chemotherapy for Advanced and Metastatic Disease

Microtubule-targeting taxane based chemotherapy is the treatment option for patients with metastatic CRPC. Taxanes, derived from molecules present in the bark of yew tress, exert potent cytotoxic effects against cancer cells via their ability to bind and stabilize interactions among β-tubulin subunits, which interact to form microtubules, a major component of the cytoskeleton. Stabilization of β-tubulin subunits prevents depolymerization of microtubules which leads to cell cycle arrest in metaphase-anaphase, and leads to apoptosis of rapidly dividing cells [32, 58]. The clinical benefit of taxanes in CRPC was first recognized in 1996 after a Canadian phase III RCT demonstrated that prednisone administration plus mitoxantrone (taxane) provided palliative benefit to 30 % of symptomatic men that led to the Food and Drug Administration (FDA) approval for CRPC [59]. Although palliative benefit was achieved there was no change in survival [59]. Results from the TAX327 and Southwest Oncology Group (SWOG) 99–16 trials established docetaxel as the first agent conferring a survival benefit in men with mCRPC. The first TAX327 study revealed an improved overall survival for docetaxel administration every 3-wks plus prednisone versus mitoxantrone plus prednisone (median overall survival 18.9 mos vs. 16.5 mos respectively) [60]. TAX327 survival improvements were revisited in a 2008 study, demonstrating similar results: 19.2 mos for docetaxel every three weeks plus prednisone versus 16.3 months for mitoxantrone plus prednisone [61]. These results were similarly observed in the SWOG 99–16 trials, revealing a 2–3 month improvement in median survival with docetaxel versus mitoxantrone [59]. Progression after first line chemotherapy is inevitable in CRPC patients with a median PFS for patients treated with docetaxel of 7.5 months, before resistance emerged [61]. After progression on docetaxel, second line cytotoxic therapy with Cabazitaxel, a second generation taxane, is initiated. Cabazitaxel shares a mechanism of action similar to docetaxel, however, a rational approach to its design resulted in bulkier side chains preventing it from being utilized as a substrate for the multi-drug resistance P-glycoprotein efflux pump, which contributes to docetaxel resistance in CRPC [61]. The pivotal trial determining the approval of Cabazitaxel was the phase III multi-national TROPIC trial examining Cabazitaxel versus mitoxantrone after resistance to docetaxel; there was a 2.4 month median overall survival advantage for Cabazitaxel, and that secured the approval of the taxane for clinical use by the FDA in 2010 [62].

AR Transport by Microtubules: Value of "Cargo" Targeting

Efforts to identify the mechanisms of resistance to docetaxel in CRPC have provided insight into novel actions of taxanes. While it is well established that taxanes inhibit the cell cycle by preventing transition between metaphase-anaphase in rapidly dividing cells in vitro, it is argued that this action alone does not account totally for clinical action in in vivo models, in which prostate cancer cells characteristically divide slowly [63]. Multiple studies now demonstrate that taxanes inhibit AR signaling in addition to inhibiting the mitotic process. Microtubules efficiently mediate transport of multiple substances intracellularly, playing a role in critical endocrine signaling pathways [64]. Once AR conformational changes occur after ligand binding, the dimerized AR/ligand complex forms and must be physically transported to the nucleus. Work by Zhu et al. revealed that ATP dependent transport along microtubules facilitated AR nuclear translocation [65]. Moreover analysis of clinical specimens from patients treated with docetaxel revealed a significant reduction in nuclear AR compared to untreated patients (38% vs. 50 % respectively), which coincided with a marked increase in cytoplasmic AR in these treated patients [65]. Work by others confirmed these initial findings by our group, demonstrating that paclitaxel substantially influenced the AR cytoplasmic/nuclear localization ratio, reducing the percentage of cells with nuclear AR (70 % to less than 30 %) [63]. This effect is dependent upon stabilized, non-mutated microtubules and directly coincides with a dose dependent inhibition of AR transcriptional activity. Full length AR association with the microtubule associated motor protein dynein was inherent and increased upon ligand induced AR translocation, navigating its nuclear transport [63]. Both docetaxel and paclitaxel have been shown to induce nuclear accumulation of forkhead box O1 (FOXO1), a potent repressor of AR transcriptional activity [66]. FOXO1 can inhibit AR by binding and sequestering it in the nucleus thus rendering it unable to activate AREs [58, 66]. Mechanistically the ability of FOXO1 to inhibit both androgen-dependent and androgen-independent AR transcriptional activity, is highly significant in defining the impact of microtubule-targeting chemotherapy on therapeutic resistance in CRPC via targeting AR variants [58, 67]. The recognition that microtubule–targeting taxane chemotherapy can also inhibit AR signaling via disruption of microtubule transport together with intranuclear inhibition by FOXO1, has shed new light into the therapeutic value of the combination of taxanes with anti-androgens against the molecular landscape of CRPC, since they target different components of the AR signaling axis. Recent clinical evidence however revealed that in CRPC patients treated with abiraterone acetate followed by docetaxel, there was a >50 % PSA decline in only 26 % of patients (compared to 54 % in TAX327 trial) and an OS of only 12.5 months (18.9 mos OS in TAX327 trial) [68], pointing to cross-resistance. Dissection of the interactions of AR variants with the microtubule network revealed that differential association with microtubules and dynein by ARVs could affect taxane sensitivity in CRPC cells [69]. This pre-clinical study established that cells harboring ARv567 exhibited inhibition of AR nuclear

translocation in response to docetaxel, while there was no effect on ARv7 nuclear localization. Furthermore, the ARv7, unlike ARv567, lacks the hinge region and part of the NLS, which contains the minimum microtubule-binding domain, rendering it independent of microtubule binding [69].

Close examination of the second line taxane chemotherapy Cabazitaxel's effect on AR localization has yielded intriguing insights into the action of the drug. Pre-clinical studies from this laboratory demonstrated that Cabazitaxel treatment of in vivo models of advanced prostate cancer, androgen sensitive and CRPC), resulted in sustained AR nuclear localization while reducing AR activity, which contrasts directly with the observed effects of docetaxel on inhibiting AR nuclear translocation (Martin et al., Cancer Res., 2015 [70]). As illustrated on Fig. 2.1, the sensitivity of CRPC cells to Cabazitaxel is dependent on neither the AR nuclear localization nor the AR variant status, a result recently corroborated by van Soest et al. who reported that Cabazitaxel maintains a potent anti-tumor and anti-PSA effect in both enzalutamide naïve and resistant cell lines, regardless of AR nuclear localization status [71]. Further evidence from this laboratory demonstrated that Cabazitaxel induced significant multinucleation as well centrosome clustering and amplification in CRPC cell lines containing full length and variant AR. Clustering

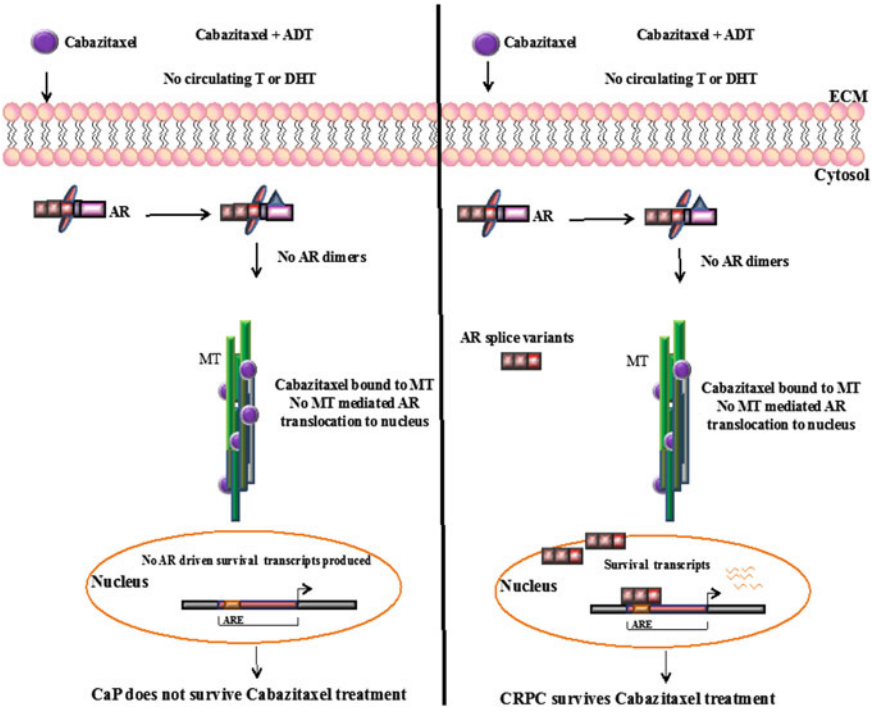

Fig. 2.1 Impact of cabazitaxel on microtubule transport network is short-circuited by the AR splice variants in CRPC

of chromosomes and supernumerary centrosomes contribute to chromosomal instability and may play a role in the regulation of the cell cycle as well [72]. The mitotic centromere associated kinesin (MCAK) and human kinesin-14 (HSET) expression are required for proper cytokinesis, and their overexpression may contribute to taxane resistance in CRPC cells via their ability to depolymerize microtubules; overexpression of both kinesins has indeed been detected in docetaxel resistant tumors [73, 74]. Cabazitaxel downregulates both kinesins, an effect coinciding with severe multinucleation and centrosome clustering, implicating cytokinesis disruption in a ligand-independent manner (Martin et al., Cancer Res., 2015 [70]). In an intriguing functional twist, androgens contribute to rapid microtubule regrowth in an AR-dependent context as suggested by evidence that androgen activates ERK signaling by complexing with the non-receptor protein tyrosine kinase Src, resulting in γ-tubulin recruitment to the centrosome and microtubule nucleation, leading to rapid regrowth of microtubules after nocodazole-induced depolymerization [75]. Moreover, functional loss of AR resulted in inhibition of microtubule regrowth in the presence of androgens, supporting the critical role for AR in promote microtubule growth from centrosome clustering [75]. One might argue that impairing AR nuclear transport and activity by microtubule targeting chemotherapy and concomitant antiandrogen therapy, may impair centrosome mediated cytokinesis adaptations that contribute to therapeutic resistance in CRPC.

Conclusions and Future Directions

The continually evolving pattern of phenotypic resistance in prostate cancer, driven by mutations and functional alterations of AR has spurred rational drug design to target both mutated and wild-type AR, as well as cross- signaling pathways interacting with AR. Crystallography-guided approaches examining the binding of various ligands and drugs to AR have been challenging to date [76]. Via ligand docking and molecular dynamics simulations, the Sawyer's group, demonstrated that drug binding to F876L mutant AR LBD leads to a lack of displacement of helix 12 due to presence of leucine at position-876, which when non-displaced assumes an agonist conformation able to recruit coactivators and drive transcription [76, 77]. The rational design of an enzalutamide analog with a bulkier B-ring moiety to prevent the agonist-like conformation of helix 12 led to DR103, that potently antagonizes F876L in prostate tumors harboring the mutation [77]. Modification of the enzalutamide backbone to reduce off-target interactions has been achieved by the novel AR inhibitor ARN509; in pre-clinical models, ARN509displayed greater anti-tumor effects than enzalutamide [78]. Rational drug design has enabled the development and testing of the novel antiandrogen ODM-201, structurally distinct from other antiandrogens, and functionally capable to fully antagonize F876L AR, as well as T877A and W741L known to confer resistance to antiandrogens [79]. ODM-201 impairs proliferation of androgen-sensitive VCaP cells overexpressing

AR more effectively than ARN-509 or enzalutamide, without crossing the blood brain barrier [79]. ODM-201 has shown good safety and tolerability profiles and significant antitumor activity (86 % PSA response in chemotherapy naïve patients) in both phase 1 and phase 2 clinical trials [80], with phase III trials ongoing (ClinicalTrials.gov, NCT02200614). Targeting of the NTD with the novel AR inhibitor EPI-001 discovered by Marianne Sadar's group is also under clinical development. The AR NTD is a relevant target for drug development due to the prevalence of ARVs lacking argetable LBD. To date, no other compound has been demonstrated to be more efficacious in targeting AR NTD and inhibiting growth of both PCa and CRPC cell lines in vitro, as well as in LNCaP xenografts [76, 81]. Development of high-efficacy EPI-001 analogs [82] is ongoing towards clinical validation of these NTD inhibitors.

This is the year 2016, marking the initiation of the precision medicine era in cancer treatment. How can anyone dispute that computer modeling of genetically altered AR structures can define new therapeutic landscapes into the effective targeting of AR signaling by attractive combination strategies of androgen agonists, antagonists and taxanes in advanced metastatic hormone–sensitive prostate cancer and CRPC? The outpouring evidence identifying clinically-relevant AR splice variants not only as therapeutic targets but also as predictive markers of advanced disease delivers promise and raises expectations. Exploitation of the rational administration of antiandrogen and chemotherapeutic agents after scrutinizing individual patient's molecular landscape for AR mutations, gene splicing, epigenetic changes, will be instrumental in maximizing efficacy and increasing survival, while minimizing the risk for emergence of treatment resistance in CRPC patients.

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Androgen Receptor Signaling in Castration Resistant Prostate Cancer

3

Yu Zhao, Donald J. Tindall and Haojie Huang

Introduction

Prostate cancer (PCa) is the second most frequently diagnosed cancer in men in the United States. Following androgen deprivation therapy, advanced PCa usually evolves into a refractory stage termed castration resistant prostate cancer (CRPC), which is responsible for most mortality. Importantly, androgen receptor (AR) signaling is still active in CRPC. Therefore, next-generation drugs that inhibit AR signaling such as enzalutamide and abiraterone are used for therapy for many patients with CRPC. These drugs provide a survival benefit but are not curative. In this article, we review the mechanisms through which the AR signaling axis promotes resistance to androgen-deprivation therapy and drives progression of CRPC. There are a number of pathways that allow AR to escape androgen-deprivation therapy, including activation of glucocorticoid receptors, synthesis of androgens in CRPC tissues, AR mutations, AR amplification and AR splice variants. Although the AR appears to be involved in resistance to other therapeutics such as the taxanes, which disrupt normal microtubule function [1], this article will focus on the role of AR in resistance to androgen-deprivation therapy.

Androgen-dependent prostate cancer (PCa) lesions usually become resistant to androgen deprivation therapy after one to three years [2], and this form of the disease is called castration resistant prostate cancer (CRPC) [2, 3]. Thus, even

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though these tumors are no longer responsive to androgen deprivation (by chemical or surgical means), they still rely on activation of the androgen receptor (AR).

AR belongs to the steroid hormone family of nuclear receptors. Other members of this family include the estrogen receptor (ER), progesterone receptor (PR), glucocorticoid receptor (GR) and mineralocorticoid receptor (MR) [4–6]. AR is an androgen-dependent transcription factor that regulates target genes in the prostate and other androgen-dependent organs. The testicular androgen testosterone (T), which is protected from degradation in the blood by its interaction with Sex Hormone Binding Globulin, enters prostate cells by passive diffusion. It is then metabolized by 5α-reductases (SRD5A) to a more active androgen, 5α-dihydrotestosterone (DHT). DHT binds to AR with high affinity ($\sim 10^{-9}$ M), causing a conformational change in the protein that results in release of heat shock chaperone proteins, interaction with importin-α, and translocation into the nucleus of a cell [7]. In the nucleus, the receptor dimer binds to androgen response elements (AREs) in enhancer and promoter regions of target genes such as prostate specific antigen (*PSA/KLK3*) and transmembrane protease serine 2 (*TMPRSS2*) [8–11]. The AR is composed of a large, disordered NH2-terminal domain (NTD), a well-ordered central DNA binding domain (DBD), and a well-ordered COOH-terminal ligand binding domain (LBD) [12–14]. The NTD harbors the Transcriptional Activation Function-1 (AF-1) domain, which contains the Transcriptional Activation Unit 1 (TAU1) domain and the TAU5 domain. TAU1, which contains the core sequence "LKDIL", is responsible for approximate 50 % of AR androgen-dependent transcription activity. TAU5, which contains the core sequence "WHTLF", is responsible for approximate 50 % of AR androgen-independent transcription activity in CRPC (Fig. 3.1) [12–14]. The DBD contains two zinc finger motifs and a short flexible hinge region. The C-terminal of AR contains the LBD and AF-2 [11–13]. AR enhances or inhibits transcription of its target genes by recruiting coactivator or corepressor proteins that alter the acetylation/methylation status of histone and non-histone proteins [15]. This leads to a relaxation and unwinding of the surrounding chromatin, thereby allowing the recruitment of components of the transcriptional machinery. Through this mechanism, the AR modulates expression of genes that are critical to PCa proliferation and survival.

Endocrine Therapies for CRPC

Given that AR plays a critical role in PCa, many studies have focused on development of new drugs targeting AR action in this disease, especially in CRPC. The three drugs discussed below are those that are most commonly used in the clinic.

Bicalutamide (casodex), which was approved for clinical use in 1995, is an effective antiandrogen with no overt agonist effects. It binds to the AR and inhibits the interaction with naturally occurring androgens. It also accelerates the degradation of AR [16]. Thus, bicalutamide prevents the activation of the AR and subsequent upregulation of androgen responsive genes [16–18]. Enzalutamide (previously known as MDV3100) has about five-fold higher binding affinity

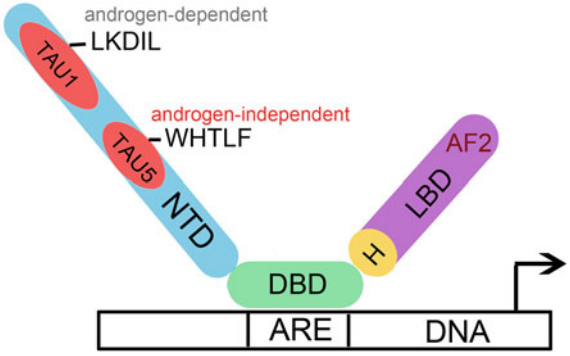

Fig. 3.1 Model of AR structure. There are three major functional domains in the AR: NH_2-Terminal Domain (NTD), DNA Binding Domain (DBD) and C-Terminal Ligand Binding Domain (LBD). The NTD contains the transcriptional Activation Function-1 (AF-1), which contains the Transcriptional Activation Unit 1 (TAU1) domain and the TAU5 domain. The core sequence "LKDIL", which is responsible for androgen-dependent AR transcription activity is in TAU1. The core sequence "WHTLF", which is responsible for androgen-independent AR transcription activity is in TAU5. The DBD contains two zinc fingers and a short flexible hinge region (H). The LBD contains the ligand binding domain and the Transcriptional Activation Function-2 (AF-2)

(IC50 ∼ 36 nM) to AR, compared to bicalutamide (IC50 ∼ 159 nM) in castration-resistant LNCaP/AR human PCa cells (engineered to express higher levels of wild-type AR to mimic the clinical scenario) [19]. Unlike bicalutamide, enzalutamide does not promote AR translocation to the nucleus, thus preventing DNA and coactivator interaction with the AR (Fig. 3.2) [19]. Enzalutamide inhibits androgen-induced expression of both *PSA* and *TMPRSS2* in LNCaP/AR cells to a greater extent than bicalutamide. Enzalutamide induces apoptosis in VCaP PCa cells with overexpressed AR, while bicalutamide does not [19].

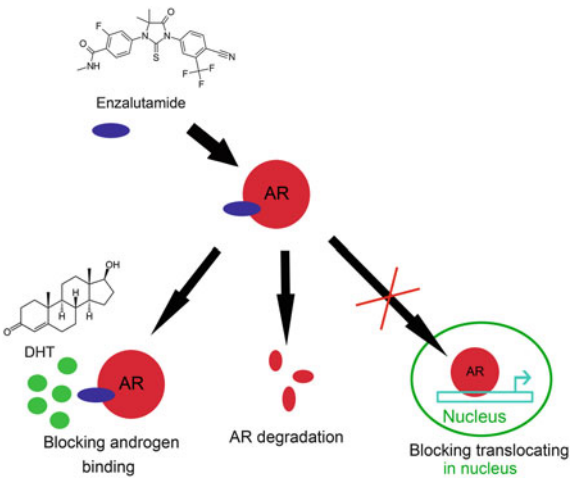

Fig. 3.2 Model of Enzalutamide inhibition of AR activity. Enzalutamide inhibits AR activity by: (*1*) Binding to AR and competing for androgen binding to AR. (*2*) Promoting AR degradation. (*3*) Inhibiting nuclear transportation of AR

Testosterone is synthesized by the Leydig cells in the testis from C21 steroids such as pregnenolone, which are generated from cholesterol. CYP17 lyase converts pregnenolone to dehydroepiandrosterone (DHEA), which is converted to testosterone through either androstenedione or Δ5–androstene-3β,17β-diol. CYP17 has both 17 alpha-hydroxylase and 17/20-lyase activities. Abiraterone inhibits 17α-hydroxylase C17/20 lyase (CYP17A1), which is expressed in the testes, adrenal gland and prostate tumor tissues [20]. Therefore, inhibiting CYP17 activity with abiraterone reduces circulating levels of testosterone and intracellular levels of DHT.

Androgen Deprivation Resistant Pathways

Although enzalutamide and abiraterone represent major breakthroughs in the treatment of metastatic CRPC, approximately 20–40 % of patients have no significant biochemical response to these agents (as measured by PSA) [21]. Among those patients who initially respond to enzalutamide or abiraterone, almost all eventually exhibit secondary resistance [21]. As discussed in detail below, there are at least six possible explanations for this resistance.

Glucocorticoid Receptor

One possible mechanism is through activation of the glucocorticoid receptor (GR) pathway [22] in CRPC. Glucocorticoids are often used in combination with chemotherapy to alleviate pain associated with cancer due to their anti-inflammatory properties and inhibitory effects on cell proliferation and angiogenesis [23, 24]. Clinical studies have shown that glucocorticoid therapy may be beneficial to patients with CRPC by suppressing synthesis of adrenal androgens and lymph angiogenesis [25, 26]. It has been shown recently that GR could be a factor that promotes resistance to enzalutamide [27]. Indeed, the structure of GR is similar to AR, and DNA binding sequences of these two receptors are very similar [28, 29]. The GR can bind to and induce many AR target genes in PCa cells by cooperating with the pioneer factor hepatocyte nuclear factor 3α (also called forkhead box factor A1, FOXA1) [30]. In addition, the transcriptional program triggered by AR and GR are overlapping in preclinical PCa models, suggesting that GR is able to promote enzalutamide resistance [22].

Synthesis of Androgens in CRPC Tissues

Another mechanism by which CRPC cells can resist androgen deprivation therapy is by synthesizing their own androgens, either by de novo synthesis from cholesterol or by metabolizing weak androgens (e.g., DHEA that is produced by the adrenal cortex).

Although DHEA at physiological concentrations can neither activate AR nor stimulate proliferation of Pca, it can be converted to T by 17β-hydroxysteroid dehydrogenase (HSD17B) and 3β-hydroxysteroid dehydrogenase (HSD3B) [31]. In CRPC tissues, both HSD17B and HSD3B are overexpressed [32]. The canonical pathway of DHT production involves T as a necessary intermediate. However, CRPC tumors can use the 5α-androstanedione pathway that avoids the need for T [33].

In addition to the classical pathway, an alternate route to DHT synthesis, called the "backdoor" pathway is utilized by CRPC cells. Thus, C21 steroids undergo 5α-reduction by SRD5A prior to being acted upon by the lyase activity of CYP17A [34] (Fig. 3.3). Interestingly, based on the increased SRD5A1 and CYP17A1 expression and activity in PCa and CRPC tissues, de novo synthesis via the backdoor pathway may be favored over the classical pathway [32]. Overexpression of many genes involved in de novo steroidogenesis and the use of adrenal androgens as substrates suggest that selective pressure by androgen deprivation therapy leads to upregulation of these enzymes and increased androgen levels in CRPC tumors [32, 35].

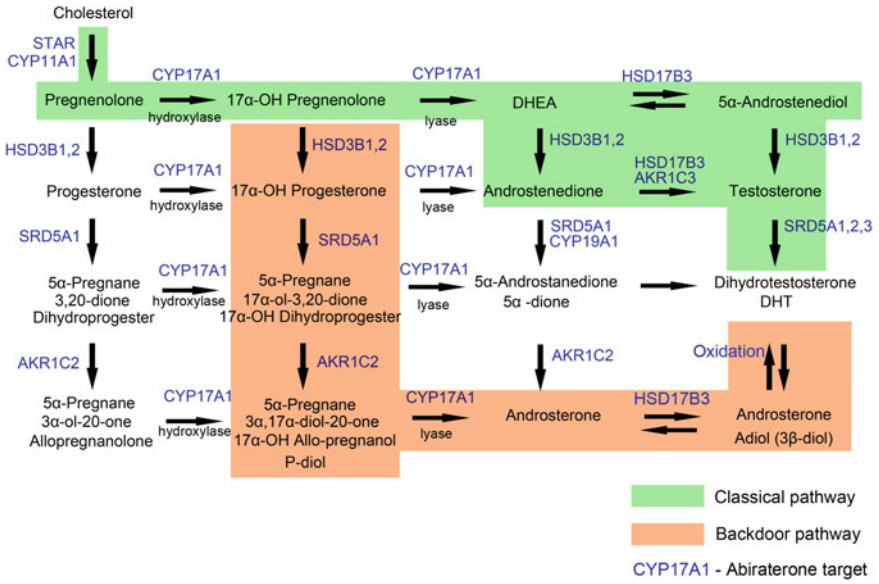

Fig. 3.3 Androgen biosynthetic pathways. In the classical pathway of androgen synthesis (*green box*), testosterone is converted to the more potent androgen DHT by the activity of SRD5A1/2. C21 precursors, such as pregnenolone, are converted to the C19 adrenal androgens DHEA and androstenedione by CYP17A1. In the "backdoor" pathway (*orange box*), C21 precursors are first synthesized by HSD3B1/2, SRD5A and AKR1C2, followed by conversions with CYP17A1, HSD17B3 and subsequent oxidation to DHT [34]

AR Mutations

Another mechanism for resisting androgen deprivation therapy is through gain of function mutations in AR. Although such mutations are rare in primary PCa, as many as 10–30 % such mutations have been reported in CRPC tissues. To date, more than 150 different mutations have been identified, most of which are located in the LBD or hinge region [36]. Typically, these mutations enhance the function of AR by increasing the recruitment of coactivators, changing the affinity and/or ligand specificity of AR, or causing an antagonist-to-agonist switch. Interestingly, antagonist-to-agonist switch mutations have been found in patients with CRPC who have been treated with hydroxyflutamide or bicalutamide [37, 38]. Recently, a point mutation, Phe876Leu in the LBD has been identified that allows AR to be stimulated by enzalutamide rather than inhibited [39–41]. This mutation of AR is found in enzalutamide resistant cell lines and xenografts [41]. Moreover, 3 out of 29 cancer patients who were treated with the enzalutamide-like drug, ARN 509, contained this mutation in their tumors [40, 42] Clinical data has revealed that both enzalutamide and ARN509 are weak AR agonist in the presence of the mutation Phe876Leu.

Amplification of AR

Another mechanism by which PCa resists androgen deprivation therapy is amplification of the *AR* gene. Approximately 30 % of androgen independent tumors exhibit an amplified *AR* gene, whereas few, if any, primary tumors exhibit *AR* amplification [43, 44]. The elevated AR expression caused by gene amplification increases the sensitivity of AR to low androgen levels after androgen ablation [45]. A clinical study reported that CRPC tumors with amplified *AR* originally responded to androgen deprivation therapy, but eventually became resistant to this therapy [43].

AR Splice Variants

One plausible explanation for resistance to androgen deprivation therapy may involve AR splice variants (AR-Vs) [46–48]. AR-Vs retain the transcriptionally important NTD and DBD core segments and exhibit constitutive transcriptional activity. However, they lack the LBD (either completely or partially) and are therefore insensitive to AR agonists or antagonists. Thus, enzalutamide, which depends on an interaction with the LBD of AR for its antiandrogenic activity cannot inhibit AR-Vs. This suggests that expression of AR-Vs may be associated with enzalutamide resistance [21, 49, 50] (Fig. 3.4). The association of CRPC with AR proteins truncated at the COOH-terminal region was first discovered in the CRPC tumor xenograft model CWR22 [51]. These studies revealed a lower molecular weight species of AR during western blot analyses using antibodies that recognized

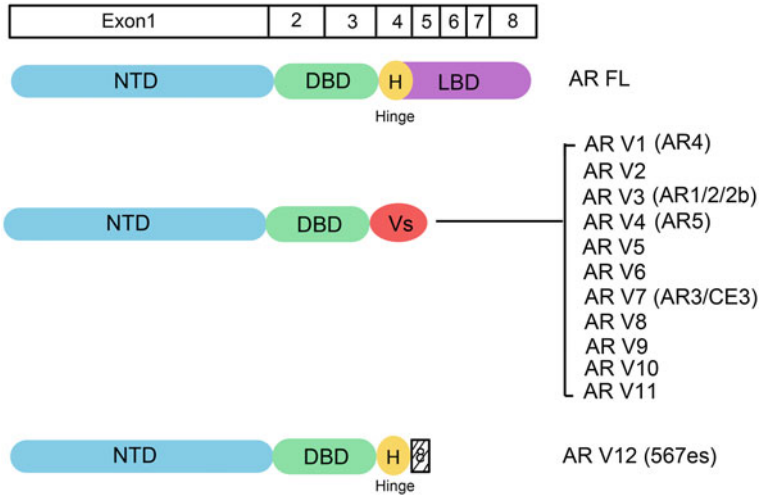

Fig. 3.4 Structures of AR full-length (FL) and AR variants (Vs). Both AR FL and AR Vs share the same NTD (*blue*) and DBD (*green*). Most AR variants lack the hinge domain and LBD. An exception is AR-v567es, which retains the hinge domain and a short piece from the LBD (exon 8)

the AR NTD, but not those recognizing the AR LBD. These low molecular weight proteins were associated with tumor progression and resistance to therapy, but were not found in the original, hormone dependent CWR22 xenografts [51]. The truncated AR protein species was thought to be a calpain-cleavage product of the full-length AR [52]. However, a small interference RNA (siRNA) study showed that siRNAs targeting AR exon 7 down regulated expression of full-length AR, but not the low molecular weight AR protein species in 22Rv1 cells, a cell line derived from CWR22 CRPC xenografts [47]. This finding suggests that the low molecular weight AR protein species is produced by different RNA species rather than by protease cleavage. This observation was further confirmed by siRNAs targeting AR exon 1 and 3'-RACE analysis. The PCR-based method showed the presence of AR variants in 22Rv1 cells and other PCa cell lines, as well as PCa xenograft models and patient specimens [53–55].

AR Variant Function

Specific knockdown of endogenous AR variants in castration-resistant cell lines such as 22Rv1 and CWR-R1, is sufficient to restore the sensitivity to androgens, overcome the resistance to enzalutamide and inhibit in vitro and in vivo growth of PCa cells under castrate conditions [49, 56, 57]. Growth of androgen-dependent LNCaP cells can be enhanced by overexpression of AR variants [47, 58]. However, there are conflicting reports regarding whether the AR variants offer an overall growth advantage, or merely maintain tumor cell viability following androgen

deprivation. For example, LNCaP xenografts expressing AR-V12/ARv567es variants alone have larger xenografts than LNCaP xenografts expressing control plasmids, but this occurs only under castrate conditions [59]. In contrast, mice engineered to express AR-V12/ARv567es in prostate epithelial cells exhibit increased prostate weights (in both castrate and intact mice) compared to full length AR controls [60]. This implies that AR-V12/ARv567es supports cell proliferation regardless of the androgen status. In addition, transgenic expression of AR-V12/ARv567es promotes more robust progression of PCa in castrate mice than that in intact mice [60]. Finally, prostates in mice expressing AR-V7 exhibit a decreased capacity to regenerate after re-administration of testosterone following castration [59]. These data indicate that AR variants may have functional roles in both normal prostate epithelium and CRPC.

Two research groups have generated transgenic mouse models that express either AR-V7 or ARv567es under the control of the prostate epithelium-specific probasin promoter [59, 60]. These models were used to study the functions of AR-Vs in vivo. The prostate-specific expression of v567es in the prostate promotes the formation of prostatic intraepithelial neoplasia (PIN) in mice at 16 weeks of age. The lesions progress further into invasive adenocarcinoma at one year of age. Microarray-based analysis of gene expression in prostate tissues from Pb-ARv567es mice suggested that the transcriptome is reprogrammed with increased expression of cell cycle related genes and genes involved in tumor initiation and progression [60]. Transgenic AR-V7 also induces PIN lesions at one year of age in approximately 50 % of mice examined, with concurrent expression of autocrine/paracrine growth factors TGFβ2 and IGF1. Overall, these studies demonstrate that AR-V proteins can promote tumorigenesis.

The Clinical Relevance of AR-Vs

A number of observations implicate the clinical relevance of AR-Vs in CRPC [47, 61]. AR-V7 protein is frequently expressed in CRPC compared to primary PCa as determined by immunohistochemistry [62]. Also, androgen deprivation results in increased AR-V expression in prostate cell lines, mouse prostate xenografts and human prostate tissues. Expression of AR-Vs is increased in CRPC compared to hormone naive bone metastases [63]. Significantly, patients with higher expression of AR-Vs exhibit a shorter duration of survival and a higher Gleason score. Transcripts for AR-V7 and AR-v567es have been detected in 100 % and 23 % of samples from bone metastases in 30 CRPC patients. Another study showed that 53 % of enzalutamide-resistant patients and 63 % of abiraterone-resistant patients exhibited detectable AR-V7 in circulating tumor cells [21]. Among men receiving enzalutamide, AR-V7 positive patients had lower PSA response rates than AR-V7 negative patients and shorter PSA progression-free survival, clinical or radiographic progression–free survival and overall survival. Overall survival was shorter in men with detectable AR-V7 at baseline than those with undetectable AR-V7 in both

cohorts. Another clinical study found a reciprocal relationship between mRNA levels of AR-Vs and the steroidogenic enzyme, AKR1C3 in CRPC bone metastases [64]. This study suggests an inverse relationship between steroidogenesis and AR-Vs. Another study demonstrated that relapsed tumors displayed increased expression of CYP17A1, full-length AR and AR-Vs in CRPC xenografts [65]. Genomic rearrangement is a key mechanism that promotes AR-Vs synthesis [55], suggesting that AR-Vs function as drivers of resistance to androgen deprivation therapy. Recently, a drug that targets the NH_2-terminal of AR was reported. EPI-001, a small-molecule antagonist of AR NTD, inhibits activation of both full length AR and AR-Vs [66]. This drug is currently being tested in the clinic.

Summary

In summary, prostate tumors can escape androgen deprivation therapy by maintaining a functional AR through various mechanisms including activation of glucocorticoid receptor, synthesis of androgens in CRPC tissues, AR mutations, *AR* gene amplification and AR splice variants. Drugs such as bicalutamide and enzalutamide target the AR LBD, and abiraterone targets the synthesis of androgens. Since all of these drugs target the LBD of AR either directly or indirectly, a big challenge will be inhibiting the NTD function in both the full-length AR and the splice variants. Thus, the AR remains an important target in our goal to develop better therapeutics against CRPC.

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Non-androgen Signaling Pathways in Castration-Resistant Prostate Cancer

4

Sivanandane Sittadjody, Thilakavathy Thangasamy, Bita NickKolgh and K.C. Balaji

Abbreviations

ADT	Androgen deprivation therapy
Akt	AKR mouse strain thymoma
AR	Androgen receptor
BAD	Bcl-2 associated death promoter
Bcl-2	B-cell lymphoma-2
BRCA1	Breast cancer susceptibility type 1
Ca^{2+}	$Calcium^{++}$
CAMs	Cell-surface adhesion molecules
CaMK	Ca^{2+}/calmodulin-dependent protein kinase
cAMP	cyclic Adenosine Mono-Phosphate
CRPC	Castration-resistant prostate cancer
Dhh	Desert hedgehog
DKKs	Dickkopf family members
ECM	Extracellular matrix

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EGF	Epidermal growth factor
EGFR	EGF receptor
EMT	Epithelial-mesenchymal transition
ET-1	Endothelin 1
ET1AR	ET-1A receptor
FISH	Fluorescence in situ hybridization
FGF	Fibroblast growth factor
FGFRs	FGF receptors
FSH	Follicle stimulating hormone
GF	Growth factor
GFRs	GF receptors
gp130	Glycoprotein 130
GPCR	G-protein coupled receptor
HER2	Human EGF receptor 2
Hh	Hedgehog
HSP 27	Heat shock protein 27
IGF	Insulin-like growth factor
IGF-IR	IGF-I receptor
IGFBPs	IGF binding proteins
Ihh	Indian hedgehog
ILs	Interleukins
IP_3	Inositol triphosphate
IWPs	Inhibitors of Wnt productions
IWRs	Inhibitor of Wnt responses
JAK	Janus kinase
JNK	c-Jun N-terminal Kinase
LPA	Lysophosphatidic acid
LRP5/6	Lipoprotein receptor–related proteins 5 and 6
MAPK	Mitogen-activated protein kinase
Mcl-1	Myeloid cell leukemia 1
mCRPC	Metastatic CRPC
MEK	MAPK/ERK kinase
MMPs	Matrix metalloproteinases
mRNA	Messenger ribonucleic acid
mTOR	Mammalian target of rapamycin or mechanistic target of rapamycin
NSAIDs	Non-steroidal anti-inflammatory drugs
NSE	Neuron-specific enolase
p42ERK	p42 (42 kDa) extracellular-signal-regulated kinase
$p70^{S6K}$	p70 (70 kDa) S6 ribosomal kinase
PCa	Prostate cancer
PGE2	Prostaglandin E2
PI3K	Phosphoinositide 3-kinase
PIN	Prostatic intraepithelial neoplasia

PIP_2	phosphatidylinositol (4,5) bisphosphate
PIP_3	phosphatidylinositol (3,4,5) trisphosphate
PKD1	Protein kinase D1
PLC	Phospolipase C
PSA	Prostate-specific antigen
PTEN	Phosphatase and tensin homologue deleted on chromosome ten
Ptch	Human pathced
Raf	Rapidly accelerated fibrosarcoma
Ras	Rat sarcoma
RGD	Arginine (R)-Glycine (G)-Aspartate (D) sequence
RIN	Ras-like protein in neurons
RKIP	Raf kinase inhibitor protein
RTK	Receptor tyrosine kinase
Shh	Sonic hedgehog
SMAD	Sma (small)/Mad (Mothers against decapentaplegic) homology
Smo	Patched of smoothened
SFRP	Secreted frizzled-related protein family
STAT	Signal transducer and activator of transcription
TGFβ	Transforming growth factor-β
TβRII	TGFβ receptor-II
TRAMP	Transgenic adenocarcinoma of mouse prostate
VEGF	Vascular endothelial growth factor
WIF1	Wnt inhibitory factor 1
Wnt	Wingless gene (Wg) homolog of int-1 (integration-1) or Wingless-related integration site

Introduction

The androgens and androgen receptor (AR) play a central role in the development and progression of prostate cancer (PCa) [1–6]. Androgen deprivation therapy (ADT) has been considered as the gold standard for the treatment of advanced prostate cancer and is universally initially effective. However, over a course of 2–3 years, almost all patients eventually become non-responsive to ADT and emerge with castration-resistant prostate cancer (CRPC) [7–9]. It is known that PCa acquires resistance through signaling molecules that could activate AR signal in the absence of androgens. While several novel anti-androgens including Abiraterone and Enzalutamide are effective in patients with CRPC, resistance to these drugs eventually ensues. The resurgence of PCa as CRPC in spite of treatment with novel anti-androgens highlights possible involvement of other non-androgen signaling mechanisms that contribute to PCa progression [10, 11]. An insight into the understanding of non-androgen signaling pathways may provide opportunities in development of novel drugs and therapies targeting CRPC. The non-androgen

signaling pathways in general, do exist in normal cells and required for their functions. Aberrant signaling could be activated by increase in ligand by autocrine, paracrine or endocrine fashion, activation of receptors by promiscuous ligands or constitutive activation. In addition, cross talk between signaling pathways also contributes to cell survival and growth. In this chapter, we have detailed major signaling pathways that play a role in PCa progression and not meant to be totally comprehensive in this ever evolving field.

Receptor Tyrosine Kinases (RTKs)

Receptor tyrosine kinases (RTK) are activated by growth factors (GFs) and insulin [12, 13]. These allosteric tyrosine kinase enzymes depend on their ligands for the activation of the kinetic domain, which phosphorylates the tyrosine moiety of their substrates leading to downstream signaling. Upon binding of GFs to their receptors (GFRs), dimerization, autophosphorylation of the receptors is followed by phosphorylation of various intracellular signaling molecules. As shown in Fig. 4.1, two major signaling pathways diverge from the RTKs namely the mitogenic (Ras/Raf/MEK/ERK) pathway and survival (PI3 K/Akt/mTOR) pathway [14, 15]. Development and progression of tumors from normal epithelial cells are generally associated with the alterations in the GFR signaling at various levels: (a) the levels of ligands; (b) levels of receptors; (c) receptor activation status and (d) intracellular signaling molecules. Upregulation or overexpression of human epidermal growth factor (EGF) receptor 2 (HER2) and EGF receptor (EGFR) in metastatic prostate tumors are good examples of this [16]. The commonly identified alterations in GFR signaling pathways in prostate cancer are as follows: (a) EGFR and its ligands; (b) insulin-like growth factor (IGF) system components (IGFs- IGF-I and -II, their receptor-IGF-IR, binding proteins-IGFBPs, and IGFBP protease systems); (c) ErbBs; (d) fibroblast growth factor (FGF) receptors FGFRs and their ligands; (e) transforming growth factor-β (TGFβ) and its receptor [1, 17, 18].

While Ras/Raf/MAPK pathway has been shown to be altered only in 43 % of primary PCa, the aberration is detected in 90 % of metastatic lesions [19]. Since Ras plays a central role in the signal transduction of several growth factors, its mutation and/or overexpression would transform a normal cell into a cancerous one. Yet, gene mutation of Ras is very uncommon among prostate cancers indicating that some other factors are involved in the increased intracellular levels of Ras [20]. Introduction of activated v-H-Ras into androgen-dependent LNCaP cells enables them to grow independent of androgens, thus signifying that activation of Ras can move PCa cells towards androgen-independence and malignant phenotype [21, 22].

Additionally, the most common alteration in Ras/Raf/MAPK pathway in terms of Raf molecule in PCa is gene rearrangement, leading to over expression of Raf. Similar to Ras, the alteration of Raf is predominant in metastatic cancers versus compared to only 1 % in primary PCa. There has been a correlation between Raf-1 expression and proto-oncogenes (c-fos and HER-2) in androgen-insensitive prostate cancers. In

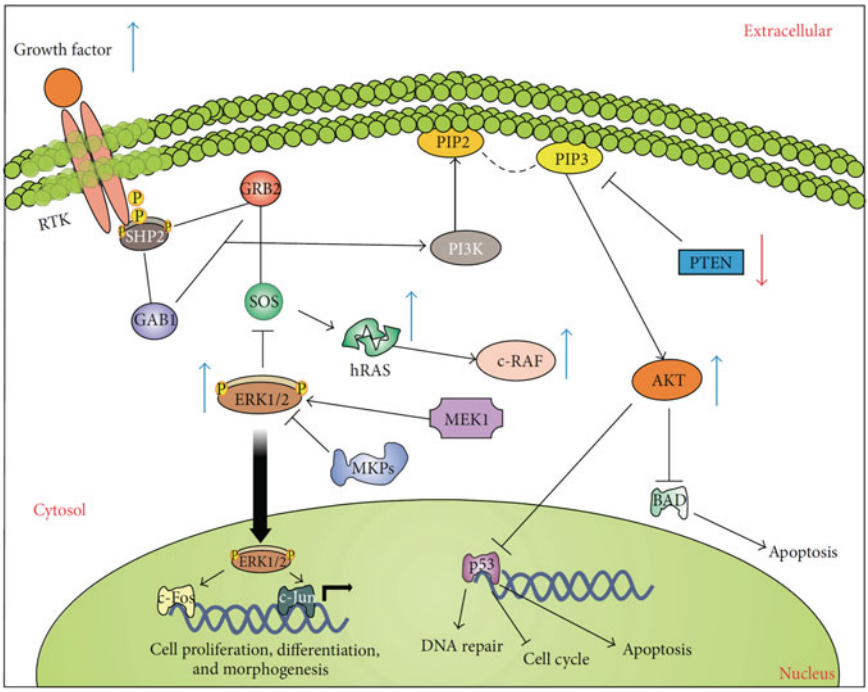

Fig. 4.1 Overview of ERK and PI3K activation and their crosstalk. The binding of the ligand to RTK dimerizes and activates the receptor, leading to the recruitment of multiple Grb2 and Shp2 molecules, which further leads to the binding of a second anchoring protein Gab1 to the complex and to the activation of Son of Sevenless (SOS). This event leads to the activation of Raf/Ras/MEK/ERK pathway. Once phosphorylated, ERKs also phosphorylate a great number of substrates present in both nucleus and cytoplasm. In the nucleus, ERK phosphorylates a series of transcription factors including Elk1, c-Fos, p53, Ets1/2, and c-Jun, each one acting as regulators of cell proliferation, differentiation, and morphogenesis. The recruitment and activation of Grb2 and Shp2 also leads to the recruitment of another docking protein, Gab1. Once phosphorylated, Gab1 recruits PI3 K to the membrane, where it phosphorylates the inositol ring of PIP-2 into PIP-3. PIP-3 facilitates the phosphorylation of AKT, which in turn regulates the activity of p53 and BAD. *Blue* and *red arrows* indicate up- and downregulated proteins in PCa, respectively. Adapted from da Silva et al. [14] with permission from Hindawi Publishing Corporation

addition, patients with increased levels of Raf-1 in PCa tissue were observed to have a significantly shorter biochemical relapse time [23]. On the contrary, in patients whose primary tumors expressed high Raf kinase inhibitor protein (RKIP) levels, the 7-year PSA recurrence rate was <10 %; whereas in patients with tumors with low RKIP expression the recurrence rate was 50 % [24, 25]. Activating Raf mutation, particularly B-Raf mutations occurs in majority of prostate cancers [26].

The important negative regulator of PI3K/Akt/mTOR pathway is *p*hosphatase and *ten*sin homologue deleted on chromosome ten (PTEN), a product of tumor suppressor gene. It impairs the activation of Akt in cells [27]. PTEN is the most frequently altered member of PI3k/mTOR/Akt pathway in PCa [28]. Homozygous deletion of *PTEN*

gene and point mutation are the commonly observed altercations in PCa samples and cell lines [29, 30]. Loss of PTEN has been reported in 9–23 % of high-grade prostatic intraepithelial neoplasia (PIN) lesions [31, 32] and 10–70 % of prostate cancer [33–39] using fluorescence in situ hybridization (FISH) analysis and this is correlated with an overall poor prognosis [39–44]. The frequency in the loss of *PTEN* is higher with high Gleason grade prostate cancers and aggressive stages [42]. While the loss of *PTEN* accounts for 10 % in hormone-dependent PCa, it is in 50 % of metastatic CRPC cases [1, 31, 32, 38, 39, 41, 43, 45–47]. Although Ras mutations are uncommon in PCa [48–50] and biallelic deletion of *PTEN* alone fails to produce any metastatic burden [51], studies including ours have shown that combination of Ras mutation along with biallelic deletion of PTEN results in aggressive metastatic PCa in mouse models [52, 53]. With the deletion of *PTEN*, Akt regulates proliferation in PCa, while AR regulates their survival, inhibition of both AR and PI3K/Akt/mTOR signaling pathways may be necessary for an effective treatment. The combination strategy has been shown to reduce the tumor growth in *PTEN*-null mice [54, 55], and similar strategy could be adapted to clinical settings.

The Ras/Raf/MEK/ERK and PI3K/Akt/mTOR pathways are considered as two major pathways that contribute to PCa survival in CRPC [56, 57]. Androgen deprived cells tend to undergo neuroendocrine differentiation as evidenced by a change in cellular morphology and expression of the chromogranin and neuron-specific enolase (NSE), and an increase in phosphorylated ERK and Akt. Therefore, inhibition of PI3K/Akt/mTOR pathway with LY294002 (PI3K inhibitor) and Rapamycin (mTOR inhibitor) suppressed the expression of neuroendocrine cell markers, whereas U0126 (MEK inhibitor) did not produce any effect [56]. Another study by Kinkade et al. [57] used a *Nkx3.1*; *Pten* mutant mouse as a preclinical model to study the effects of Ras/Raf/MEK/ERK and PI3K/Akt/mTOR inhibition on hormone-dependent and independent PCa growth [178]. When the tumors from these mice were treated with inhibitors of the two pathways simultaneously (rapamycin and the MEK inhibitor, PD0325901) in vivo and in vitro, a synergistic inhibition was noted. The effects were more pronounced in androgen-deficient mice than the androgen-intact ones proving once again that the inhibition of these pathways could be a more fruitful approach when used as a combination therapy instead of monotherapy against PCa, especially in CRPC [57]. To corroborate the mouse study the authors also studied the status of signaling molecules from these two pathways in human tumors using human patient tissue microarrays. The study reported that aberrant activation of some of the components of these pathways (Akt, mTOR, p70S6K) is frequent in advanced prostate tumors. Moreover, there is concordant activation of the Ras/Raf/MEK/ERK pathway in high percentage of these tumors [57].

TFG-β/SMAD Signal

Transforming growth factor (TGF)-β1 is a multifunctional GF that regulates various functions of cells including proliferation, extracellular matrix (ECM) production and degradation, cell differentiation and apoptosis. In normal prostate, TGF-β has

been shown to have tumor-suppressor-like function by inhibiting epithelial cell proliferation and stimulating apoptosis [58, 59]. The signaling pathway is depicted in Fig. 4.2. It is well established that TGF-β1 is a physiological regulator of prostate growth through its ability to inhibit proliferation and induce apoptosis and TGF-β is overexpressed in CRPC [60–63]. Despite of the fact that there is an upregulation of

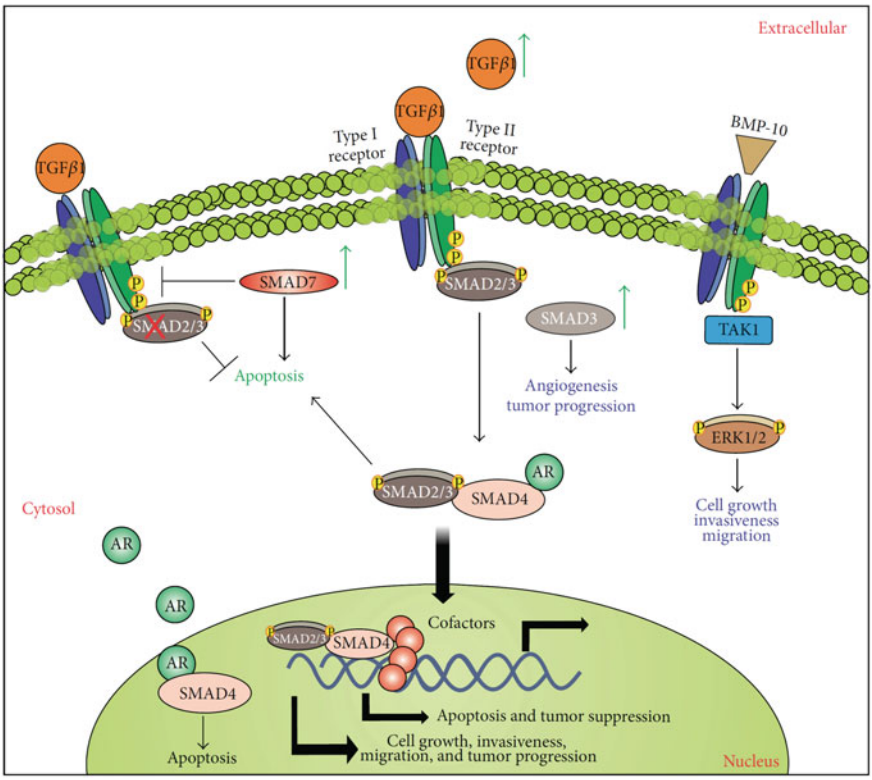

Fig. 4.2 The TGF-β/SMAD signaling pathway and its implication in prostate cancer. When a TGF-β ligand binds to the constitutively active type II receptor, this complex associates with the type I receptor, forming a tetrameric receptor. The type II receptor phosphorylates and activates the type I receptor, which allows the recruitment of R-SMADs. The activated type I receptor then phosphorylates the MH2 domain of R-SMAD, activating it. Activated R-SMADs form complexes with SMAD4, which is then translocated to the nucleus. In the nucleus, SMAD complexes interact with nuclear proteins to activate or repress the transcription of target genes. Furthermore, BMP-10 can signal through SMAD-independent pathways and inhibit cell growth, invasiveness, and migration. TGF-β can also promote androgen receptor (AR) translocation into the nucleus and AR-dependent gene transcription. AR can combine with SMAD4 and regulate TGF-β-mediated apoptosis. In normal epithelium or early-stage cancer cells, TGF-β is thought to act as a tumor suppressor, by inhibiting cell growth, invasiveness, and motility and promoting apoptosis. In more advanced cancer cells, TGF-β has tumor-promoting functions; it promotes proliferation, invasion, and motility of cells and inhibits apoptosis. *Green arrows* indicate potentially up-regulated proteins in PCa. Adapted from da Silva et al. [14] with permission from Hindawi Publishing Corporation

TGF-β, the expression of cellular receptor (mainly TβRII) is found to be down regulated, the combination of which leads to invasive and hormone refractory form of PCa [64, 65]. Several studies suggested that the effects of TGF-β and its signaling pathway depend on the stage of the PCa. During the early stages, the signals are directed towards growth suppression [66, 67] whereas in advanced stages signals are growth promoting [68–70].

Due to the existence of dysfunctional TGF-β signaling pathway in prostate cancers, the pathway could be a therapeutic target. Because of loss in TβRII expression, strategies restore to its expression can be explored using drugs commonly used in clinical practice [71]. Epristeride, the 5α-reductase inhibitor, not only shown to inhibit the expression of IGF-I but also increase the expression of TβRII, which suggests the existence of crosstalk between signaling pathways of IGF and TGF-β [72]. Similarly, Quinazoline-based drugs such as doxazosin and terazosin (α1-adrenoreceptor blockers) have been reported activate TGF-β signaling pathway [73].

JAK/STAT Pathway

The cytokines play a major role in regulating many cellular functions including proliferation, apoptosis, migration, invasion and angiogenesis and hence their expression and function have been studied extensively in the case of PCa [74, 75]. Cytokine signaling via Janus kinase (JAK)/signal transducer and activator of transcription (STAT) pathway play a major role in several cancers including metastatic CRPC (mCRPC) as depicted in Fig. 4.3. Among various cytokines, interleukins (ILs) in particular IL-6 has been reported to be a pleiotropic cytokine involved in prostate regulation and in PCa development/progression. Hence, the signaling pathway of IL-6 has been discussed here as an example representing JAK/STAT pathway of ILs.

The IL-6 levels are significantly higher in the plasma and serum samples from patients of CRPC [76, 77] and metastatic PCa [78–82]. Several groups studied the involvement of IL-6 and its signaling pathway in the development and progression of PCa using benign prostate cells, PCa tissues and PCa cell lines. In benign prostate cells the expression of gp130, the signal transduction subunit of their receptors, was confined to the epithelial and stromal cells, while IL-6 was immuno-localized predominantly in epithelial cells [83, 84]. Similarly, in PCa tissues, the gp130 was immuno-localized in both stroma and epithelium, and is over expressed with the increasing Gleason grade. However, IL-6 was detected in all cell types, which is also over expressed with increasing Gleason grade [83]. In fact, IL6 is currently used as a predictor in clinical nomograms [85–88].

A member of STAT family, STAT3 has been reported to be constitutively active in many cancers including prostate cancers [89, 90]. STAT3 is known to play multiple roles in the progression of prostate cancer [89]. One of the significant manifestations associated with the constitutively active STAT3 is the expression of breast cancer susceptibility type 1(BRCA1) protein in PCa cells [91]. BRCA1 interaction with JAK1/2 leads to the activation of STAT3 by phosphorylation resulting in the

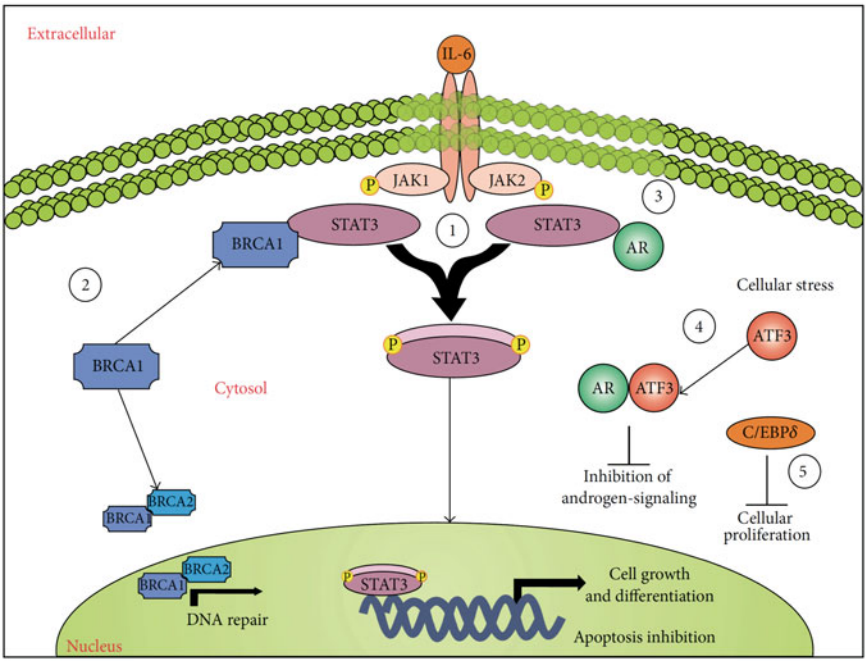

Fig. 4.3 The JAK/STAT signaling in prostate cancer. *1* The JAK/STAT pathway has been found constitutively activated in PCa cells, leading to induction of tumor cell proliferation and apoptosis inhibition mediated by STAT3 activation. *2* BRCA1/2 is required for DNA repair in normal cells. However, in PCa, BRCA1 can bind STAT3 to promote JAK/STAT3 activation. *3* AR is a well-characterized cross-talk pathway in PCa. When activated, AR can bind to STAT3 leading to the activation of JAK/STAT cascade, being important in the induction of cell proliferation and apoptosis inhibition. *4* Under stress conditions, ATF3 is activated and plays a crucial role in the maintenance of cell integrity and homeostasis. ATF3 does so by interacting with AR, leading to inhibition of androgen signaling and, consequently, the inhibition of cell proliferation. However, ATF3 is downregulated in PCa cells, suggesting that this pathway provides an important mechanism of defense against cancer. *5* Similarly, C/EBPδ is required to inhibit cell proliferation by binding to STAT3. Nevertheless, C/EBPδ is typically downregulated in PCa, and, therefore, it could be used as an strategy in the development of therapeutic drugs against PCa growth. Adapted from da Silva et al. [14] with permission from Hindawi Publishing Corporation

induction of cell proliferation and inhibition of apoptosis [91]. STAT3 induces the pro-survival and anti-apoptotic genes such as Bcl-2, Bcl-xl, survivin and Mcl-1 in PCa and other tumors [90]. Additionally, STAT3 induces the expression of vascular endothelial growth factor (VEGF), a pro-angiogenic factor, which in turn is responsible for regulating various matrix metalloproteinases enzymes (MMPs) that augments the migration and invasion of tumor cells [90, 92–95]. Interestingly, high levels of STAT3 detected in both malignant and normal surrounding tissues suggesting that the activation of STAT3 may occur prior to any detectable histological changes in the prostate [96]. Furthermore inhibition of this JAK/STAT3 pathway has been reported to induce apoptosis and suppress PCa growth [97].

STAT5, another member of STAT family is also known for its tumor promoting role in prostate cancer cells in vitro and in vivo. Studies in PCa cell lines and in transgenic adenocarcinoma of mouse prostate (TRAMP) model demonstrate the pro-survival role of STAT5 in prostate cancer [98]. STAT5 was also reported to promote the metastatic behavior of PCa cells [99]. Similar to STAT3, the target genes for STAT5 include Bcl-XL and Cyclin-D1 [100]. Even though STAT5 has been shown to be constitutively activated in prostate tumors, unlike STAT3 it is not activated in the surrounding normal tissues [101]. Epithelial expression of STAT5 correlates with the Gleason grade [102, 103]. The inhibition of STAT5 A/B in prostate cancer cells has been reported to induce apoptosis and thereby reducing the tumor growth of xenografts in nude mice [104, 105]. In addition, the expression of truncated mutant of STAT5B abolished the growth of tumor cells derived from TRAMP mouse in soft agar and also reduced the xenograft growth of these cells in nude mice [106]. Therefore, targeting JAK/STAT signaling pathway can be used as another strategy in controlling proliferation of prostate cancer cells, consequently tumor growth and metastasis [107].

G-Protein Coupled Receptor (GPCR) Signaling

Several bio-molecules mediate their biological effects on normal and/or cancer cells using seven transmembrane G-protein coupled receptors (GPCRs). Some of the molecules that act as a GPCR ligand in prostate cells include (a) acetylcholine [108]; (b) angiotensin [109, 110]; (c) Bombesin [109, 111]; Bradykinin [112–114]; Endothelin-1 (ET-1) [115, 116]; Follicle stimulating hormone (FSH) [117]; Isoproterenol [118]; Lysophosphatidic acid (LPA) [119–121]; Neurotensin [122]; Prostaglandin [123]; Thrombin [124]. As shown in Fig. 4.4, activation of GPCRs by their respective ligands results in a variety of intracellular signaling pathways in normal and cancer cells [125].

Alterations in GPCR signaling pathway has been identified in PCa. First, the enzymes that regulate the expression of GPCR ligands are found to be elevated in PCa. For instance, kallikrein 2 which possesses kininogenase activity (responsible for the production of Kinins) are elevated in prostate cancer [126, 127]. In turn, the kinins (Bradykinin 1 and 2) serve as ligands for GPCRs (B1 and B2) in order to mediate their functions including cancer cell division, survival and invasion [128–131]. Second, there are several studies reporting the increased production of various GPCR ligands such as ET-1 [115, 116], FSH [132] and LPA [133, 134] in PCa cells. Third, up regulation of receptors such as orphan prostate-specific GPCR [135], bradykinin receptor 1 [112], FSH receptor [136] and ET-1A receptor (ET1AR) [115, 137] have been reported in malignant prostate specimens. Hence, in the malignant PCa tissues both the ligands and GPCRs are over-expressed thereby resulting in constant GPCR signals which contribute to the progression of the disease. Some of the GCPRs (like $ET_{1A}R$) have already been targeted in clinical trials by inhibiting the $ET_{1A}R$ signal using GPCR antagonist Atrasentan [138, 139].

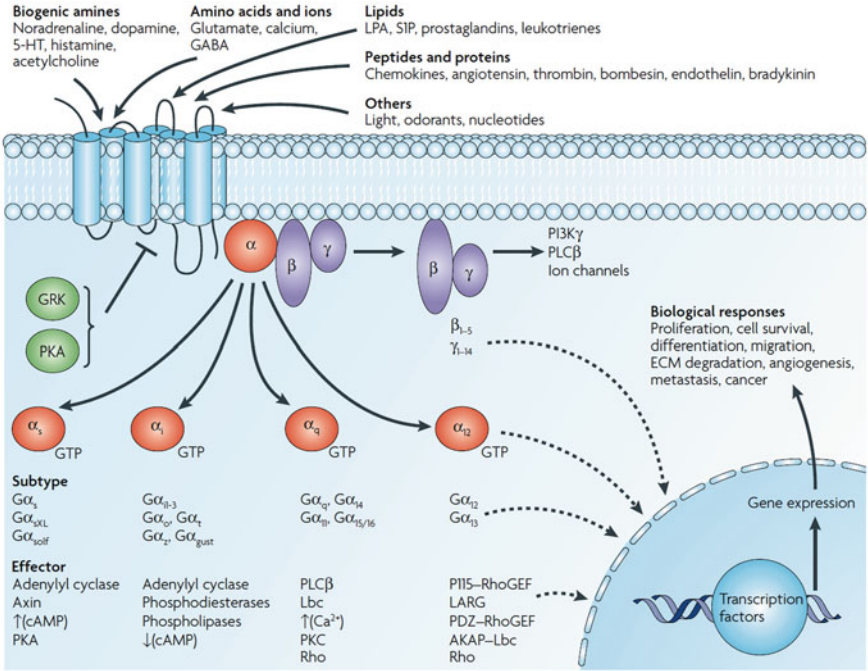

Fig. 4.4 Diversity of G-protein-coupled receptor signalling. GPCRs interact with heterotrimeric G proteins composed of α, β and γ subunits that are GDP bound in the resting state. Agonist binding triggers a conformational change in the receptor, which catalyses the dissociation of GDP from the α subunit followed by GTP-binding to Gα and the dissociation of Gα from Gβγ subunits. The α subunits of G proteins are divided into four subfamilies: Gαs, Gαi, Gαq and Gα12, and a single GPCR can couple to either one or more families of Gα proteins. Each G protein activates several downstream effectors. Typically $G\alpha_s$ stimulates adenylyl cyclase and increases levels of cyclic AMP (cAMP), whereas $G\alpha_i$ inhibits adenylyl cyclase and lowers cAMP levels, and members of the $G\alpha_q$ family bind to and activate phospholipase C (PLC), which cleaves phosphatidylinositol bisphosphate (PIP_2) into diacylglycerol and inositol triphosphate (IP_3). The Gβ subunits and Gγ subunits function as a dimer to activate many signalling molecules, including phospholipases, ion channels and lipid kinases. Besides the regulation of these classical second-messenger generating systems, Gβγ subunits and Gα subunits such as $G\alpha_{12}$ and $G\alpha_q$ can also control the activity of key intracellular signal-transducing molecules, including small GTP-binding proteins of the Ras and Rho families and members of the mitogen-activated protein kinase (MAPK) family of serine-threonine kinases, including extracellular signal-regulated kinase (ERK), c-jun N-terminal kinase (JNK), p38 and ERK5, through an intricate network of signalling events that has yet to be fully elucidated. Ultimately, the integration of the functional activity of the G-protein-regulated signaling networks control many cellular functions, and the aberrant activity of G proteins and their downstream target molecules can contribute to cancer progression and metastasis. *5-HT* 5-hydroxytryptamine; *ECM* extracellular matrix; *GABA* gamma-aminobutyric acid; *GEF* guanine nucleotide exchange factor; *GRK* G protein receptor kinase; *LPA* lysophosphatidic acid; *PI3 K* phophatidylinositol 3-kninase; *PKA* and *PKC* protein kinase A and C; *S1P* sphingosine-1-phosphate. Adapted from Dorsam and Gutkind [125] with permission from Nature Publishing Group

Signaling by Cell-Surface Adhesion Molecules (CAMs)

To date there are about 50 CAMs have been identified that mediate the cell-cell or cell-ECM interaction and belong to one of the four following families of proteins: (a) integrins, (b) cadherins, (c) IgCAMs (immunoglobulin superfamily) and (d) selectins [140, 141]. In addition to their role in attachment and interaction with ECM, some of these CAMs actively participate in signal transduction that governs various cellular events such as cell survival, proliferation, differentiation, epithelial-mesenchymal transition (EMT), motility, migration and apoptosis [142] as shown in Fig. 4.5.

Integrins are required for interaction of prostate basal cells with the surrounding stroma, influence various functions (like growth, survival and differentiation) and therefore expressed in normal prostate tissue. The predominant types of integrin include α2β1, α53β1, α6β1, α3β1, αvβ3 and α6β4 [143–148]. Integrins such as αvβ3, α2β1, α3β1 and α6β1 are involved in the progression of cancer whereas α4β1 is associated with tumor suppression. In PCa, the expression profiles of integrins are altered leading to proliferation, migration and metastasis. Compared to cancer cells, P69 (normal cell line) showed higher expression of integrin subunits strengthening the fact that those cells with strong adhesion were hindered from migration. Similarly, in normal tissue α6β4 is involved in the formation of hemi-desmosomes which links laminin of ECM to the cytoskeleton [149]. However, in PCa the expression of α6β4 is often reduced, thus leading to loss of hemi-desmosomes and resulting in weak cell-cell adhesion [147]. Interestingly, the expression of α6 integrins in prostate tumors has been found to be reduced or absent, which correlates with the invasiveness of the cancer cells [150]. On the contrary, when aggressive bone metastatic PCa cell line C4-2B were compared to their parental cell line LNCaP (derived from lymph node), no changes were seen in the expression of integrin subunits. Instead, the combination of integrin heterodimers in these cells gets altered during the transformation into aggressive cell lines. The α2β1 heterodimers found in C4-2B cells were absent in LNCaP cells [141].

Disruptions in the expression and functions of cadherin and β-catenin play a central role in the alterations of prostate cancer cell adhesion, migration and invasion. Loss or downregulation of E-cadherin expression has been described in several tumors including prostate cancers [151–157]. The tumor samples from the patients of higher grade prostate cancer with a Gleason score ≥8 have been reported to have lower E-cadherin and higher expression of N-cadherin compared to the samples obtained from lower grade prostate cancer patients [152, 158, 159]. In conjunction with these findings, the decreased expression of β-catenin was also found to be associated with the decline in E-cadherin level and correlated with higher grade prostate cancer [160]. Supporting these clinical reports, in vitro studies also demonstrated that there is decline in the expression of E-cadherin with the invasive nature of cancer cell lines [161] or invasive rat prostate tumors [162].

It is evident that CAMs signaling pathways may be useful targets to interfere with migration and invasion of PCa cells. An earlier study by Humphries et al. [163] showed that blocking integrins using RGD peptides interfered with the invasion of

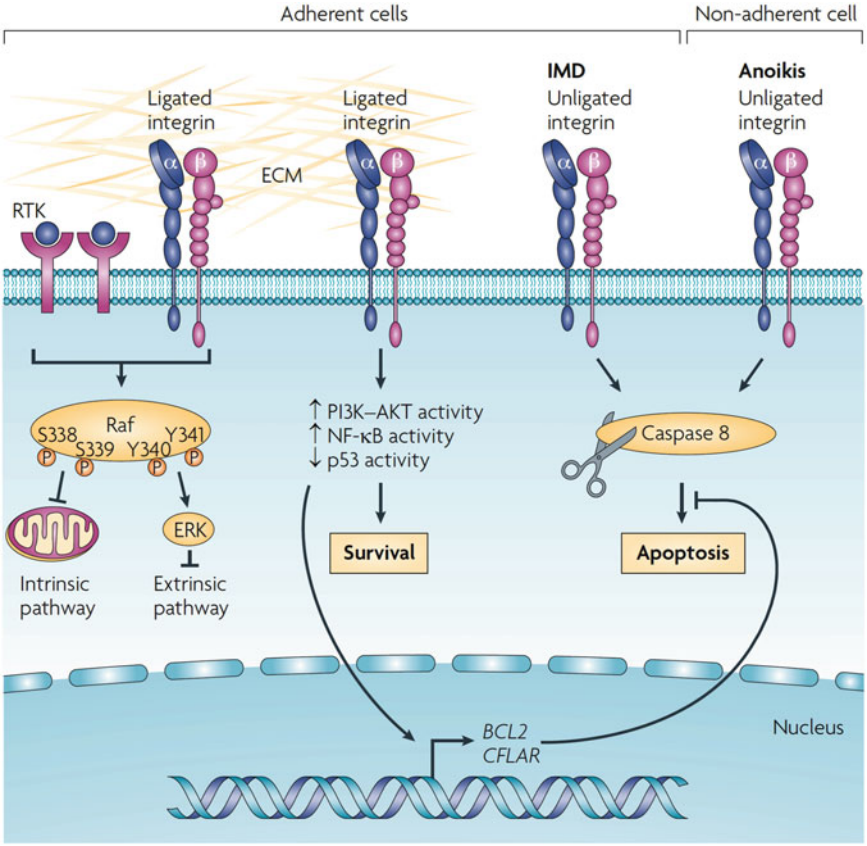

Fig. 4.5 Integrins role in pro-survival as well as pro-apoptotic signals. The balance between pro-survival and -apoptotic pathways depends on the ligation status of the surface integrins expressed by a given cell. In a cell in which most of the integrins are ligated, a pro-survival pathway is initiated through increased nuclear factor-κB (NF-κB) or PI3 K–AKT activity, decreased p53 activation and increased expression of the pro-survival molecules BCL-2 and FLIP (also known as CFLAR). Cooperative signaling between growth factor receptors and integrins also differentially activates Raf leading to distinct mechanisms of cell survival. Signaling through integrin αvβ3 and the fibroblast growth factor receptor promotes phosphorylation of Ser338 and Ser339 of Raf, protecting cells from the intrinsic pathway of apoptosis; integrin αvβ5 and VEGF receptor 2 phosphorylate Tyr340 and Tyr341 of Raf, preventing apoptosis through the extrinsic pathway. In adherent cells with several uligated integrins, the unligated integrins initiate cleavage of caspase 8, triggering apoptosis through integrin-mediated death (IMD). On complete loss of adhesion, cell death is initiated through a process termed anoikis. Apoptosis induced by anoikis may proceed through either the intrinsic or extrinsic pathways. *ECM* extracellular matrix; *RTK* receptor tyrosine kinase. Adapted from Desgrosellier and Cheresh [142]. With permission from Nature Publishing Group

cancer cells in vitro and metastasis in mouse model. Subsequently, several synthetic peptides containing RGD sequence or other integrin binding sequences, non-peptide RGD mimetics and disintegrins (the integrin-binding proteins extracted from viper snake venom) have been shown to suppress cancer cell metastasis in experimental models and in animal models [164]. In the context of cancer treatment targeting integrins, several drugs have entered clinical trials [142, 165, 166]. Vitaxin, later developed into etaracizumab (anti- αvβ3 antibody) was among the first to enter clinical trial targeting αvβ3 integrins in prostate cancer [167–170]. An αv antibody, CTNO 95 targeting αvβ3 and αvβ5, has also been tested in phase I and II clinical trials, and found to have little toxicity and some anti-tumor activity [171].

Wnt/β-Catenin Pathway

Wnt signaling regulates the self-renewal of prostate cancer cells with stem cell characteristics independent of AR [172, 173]. The expression of several Wnt ligands were found to be altered in advanced prostate cancer cells. The involvement of Wnt signaling in regulating β-catenin has been depicted in Fig. 4.6. High levels of both Wnt1 and β-catenin have been reported to be associated with advanced, metastatic, hormone-refractory prostate carcinoma whereas normal prostatic tissue failed to exhibit any detectable nuclear staining of β-catenin [174]. Wnt1 has been shown to be elevated in some human prostate tumor tissues, lymph node and bone metastases and positively correlated to Gleason score and cellular level of β-catenin and PSA [175]. Over expression of other Wnt ligands like Wnt2 and Wnt5 was also found in PCa. It is suggested that overexpression of Wnt5a activates Wnt/Ca^{2+} pathway through CaMK2 in PCa which might induce cytoskeleton reorganization and increased cell motility, and subsequently stimulation of invasion activities [176].

Wnt inhibitors are secreted proteins that block Wnt signaling either by binding to Wnt themselves (SFRP family and WIF1) or to the LRP5/6 Wnt co-receptors (Dickkopf family, DKKs). Down regulation of these Wnt inhibitors occurs frequently in human cancers due to promoter hypermethylation [177] and down regulation of both WIF1 and SFRP1 are reported in PCa [178]. In vivo experiments has shown that stable DKK1 (inhibitor of Wnt pathway) blocked Wnt induced osteoblastic activity. Prostaglandin E2 (PGE2) secreted by osteoclasts can increase Wnt inhibitor expression by both PCa and osteoblast lineage cells in the early osteolytic phase of PCa bone metastasis. PGE2 showed to exert a biphasic effect on the expression of LRP5/6, β-catenin. At low dose it increases expression of LRP5/6, β-catenin in MC3T3 cells, whereas at higher dose it inhibits the expression of LRP5/6. However, PGE2 increased the expression of other two soluble Wnt inhibitors Dkk1 & SFRP-1 [179, 180].

Several groups have identified various drugs and phytochemicals that either directly or indirectly disrupt β-catenin-mediated Wnt signaling. These agents include non-steroidal anti-inflammatory drugs (NSAIDs), exisulind, vitamin A derivatives, endostatin and phytochemicals such as flavonoids (genistein), retinoids and lycopene. Two class of small molecules have been shown to disrupt Wnt

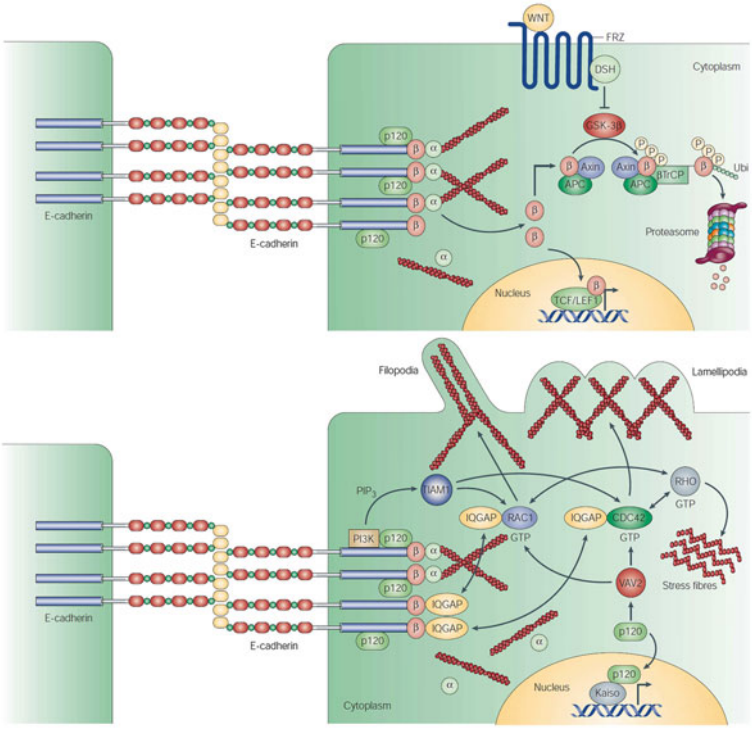

Fig. 4.6 E-cadherin and Wnt signaling pathways. **a** After loss of epithelial (E)-cadherin function and disassembly of the cytoplasmic cell-adhesion complex (CCC), catenins are released and accumulate in the cytoplasm. β-Catenin (β) is then sequestered by the adenomatous polyposis coli (APC)–axin–glycogen synthase kinase 3β (GSK-3β) complex and phosphorylated by GSK-3β. Phosphorylated β-catenin is specifically bound by βTrCP, a subunit of the E3 ubiquitin-ligase complex, which ubiquitylates β-catenin and thereby earmarks it for rapid proteosomal degradation. However, on activation of the Wnt signalling pathway, GSK-3β is repressed and β-catenin is no longer phosphorylated. It translocates to the nucleus where, together with the TCF/LEF1 transcription factors, it modulates the expression of several target genes that are known to be involved in cell proliferation and tumour progression. **b** Cytoplasmic p120-catenin (p120) activates the RHO-family GTPases RAC1 and CDC42 (probably through the RHO guanine-nucleotide exchange factor (RHO-GEF) VAV2) and represses RHO by an unknown mechanism. Phosphatidylinositol 3-kinase (PI3 K) is recruited to the membrane by intact E-cadherin adhesion junctions, where it generates phosphatidylinositol-(3,4,5)-triphosphate (PIP3), resulting in the activation of the RHO-GEF TIAM1 and subsequently of RAC1 and CDC42. GTP-bound, activated RAC1 and CDC42 sequester the GTPase-activating protein IQGAP1, which in its free form would otherwise bind to β-catenin, thereby displacing α-catenin (α) from the CCC and disrupting the anchoring of the CCC to the cytoskeleton. Together, these activities affect the organization of the actin cytoskeleton, and possibly the migratory behaviour of tumour cells, as follows: activated CDC42 induces the formation of filopodia; activation of RAC1 results in the formation of lamellipodia; and activated RHO induces the formation of actin stress fibres. Cytoplasmic accumulation of p120-catenin can result in its translocation to the nucleus, where it associates with the transcription factor Kaiso and modulates gene expression. However, the functional implications of these changes in gene expression for tumour progression are not known. DSH, dishevelled; FRZ, frizzled; Ubi, ubiquitin. Adapted from Cavallaro and Christofori [173] with permission from Nature Publishing Group

pathway responses; (a) benzothioazole-based inhibitors of Wnt productions (IWPs), target the activity of Porcupine, a membrane-bound acyltransferase that is essential to the production of Wnt proteins, (b) the other class is inhibitor of Wnt responses (IWRs) that abrogates destruction of AXIN proteins. XAV939 is another small molecule derivative that could selectively inhibits β-catenin mediated transcription and also stimulate β-catenin degradation by stabilizing AXIN. Although these small molecules have been suggested as promising next generation chemotherapy, the clinical use of these agents is associated with certain risks and challenges. It is possible that chemical modulators of these developmental pathways will have unintended effects on tissue homeostasis and regeneration [181]. Targeting specific Wnt proteins and receptors that are aberrantly overexpressed in tumors using blocking antibodies may also be an attractive strategy for targeting Wnt signaling in cancer cells. For example intraperitoneal injections of WNT3A—neutralizing antibodies have been shown to decrease proliferation and induce apoptosis in a mouse model of prostate cancer [182].

Given the fact that deregulation of Wnt signaling pathways is insufficient to induce tumor formation, it is unlikely that inhibition or activation of Wnt signaling pathway as monotherapy would be sufficient to halt cancer progression. Activation or inhibition of Wnt signaling can either sensitize or desensitize cancer cells to toxic insults, which might be advantageous in the development of combination therapies(14).

Hedgehog Pathway

The Hedgehog (Hh) signaling system is another developmental signaling pathway which is involved in maintenance of stem cell population, tissue repair and regeneration in normal adult tissues. A brief outline of this signaling pathway has been provided in Fig. 4.7. The Hh proteins aid in various processes of embryonic development including cell growth, cell differentiation, patterning and organogenesis [183–186]. Aberrant or uncontrollable activation of this signaling system is a feature of many cancer types because of its role in EMT leading to metastasis.

The Hh signaling is controlled by multiple steps at different subcellular levels that are involved in several regulatory mechanisms unique to the pathway. In humans, three Hh ligands, Sonic hedgehog (Shh), Indian hedgehog (Ihh), and Desert hedgehog (Dhh) are widely expressed in tissues among which Shh is best characterized [187]. The Hh-pathway increases metastasis by promoting expression of snail protein and by reducing expression of E-cadherin. Also, Shh increases the angiogenic factor angiopoietin-1, decreases angiopoietin- and antiapoptotic genes, and increases cyclins (D1 and B1) and proapoptotic genes, like Fas [188, 189]. Studies suggests that the Hh pathway is activated in human prostate cancers [183, 190] and its signaling is active in the epithelium of the urogenital sinus from where the prostate is derived [191]. Expression of Shh, Smo and Ptch, markers of the Hh signaling pathway activation, are also increased in cancerous compared to normal prostate epithelium [190]. Higher mRNA and protein expression of several Hh

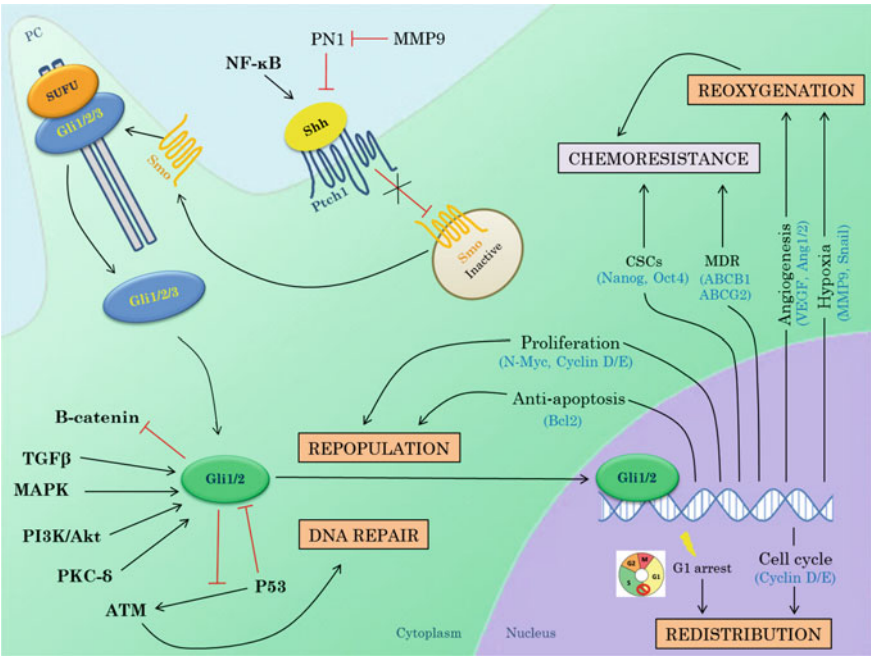

Fig. 4.7 Hedgehog signaling and rationale for combination therapy with (chemo) radiotherapy. Upon Sonic Hedgehog (Shh) ligand binding to its receptor Patched (Ptch1) 1, the repression of Smoothened (Smo) is relieved, resulting in the movement of Smo from the intracellular vesicles to the primary cilium. Smo becomes activated and promotes the activation of the Gli proteins (Gli1/2) that enter the nucleus and promote transcription of the target genes (canonical pathway activation). The Gli transcription factors can also become activated by means of non-canonical pathway activation due to significant crosstalk with other important pathways such as the PI3K-Akt, KRAS, PKC-δ and TGFβ pathways. The Hh signaling also has important interactions with Wnt pathway and P53. The response to radiation therapy is determined by the four R's of radiobiology: repopulation, repair of sublethal DNA damage, redistribution and reoxygenation. Hh signaling can potentially interfere with all these processes and targeting Hh signaling could therefore increase radiosensitivity of tumor cells. Moreover, inhibition of Hh signaling could also improve the response to chemotherapy by targeting multidrug resistance and cancer stems cells in addition to its effects on tumor vasculature. Abbreviations: *PC* primary cilia; *MDR* multidrug resistance; *CSCs* cancer stem cells. Adapted from Gonnissen et al. [186] with permission from MDPI AG

family members were observed in prostatectomy specimens and the levels correlated with poor prognostic features such as larger tumor size, higher pretreatment PSA level advanced stage [192]. Preclinical models suggest that inhibition of Hh signaling may improve therapeutic outcomes in human prostate cancer [193, 194]. A randomized phase II study of itraconazole an antifungal drug, revealed its ability to inhibit Hh signaling in men with CRPC [195].

Protein Kinase D1 Signaling

Protein kinase D1 (PKD1) belongs to calcium/calmodulin-dependent protein kinase (CaMK) family [196] and is down regulated in advanced PCa [197]. The signaling pathway is depicted in Fig. 4.8. Activated PKD1 has been reported to be a modulator of several kinase-mediated signal transduction pathways such as p42ERK [198]. Recent works showed that PKD1 can phosphorylate RIN, a regulator of Ras function [199]. Interestingly the phosphorylated site on RIN by PKD1 involves in the interaction with 14-3-3. Therefore, it has been presumed that by phosphorylating RIN, PKD1 favors its sequestration with 14-3-3 and relieving its inhibition on Ras pathway. In contrast, PKD1 inhibits the JNK signaling pathway by EGFR. Phosphorylation of EGFR on two distinct sites was reported to be critical for this inhibition and PKD1 is

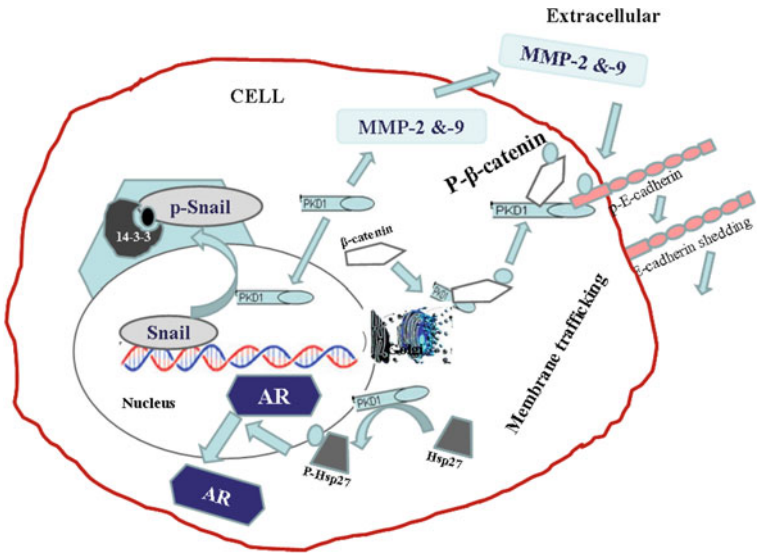

Fig. 4.8 Schematic representation of signaling pathways modulated by Protein Kinase D1 in cancer. The schematic representation shows the pathways that activate PKD1 in prostate cancer cells. Activated PKD1 is rapidly translocated from the membrane to the cytoplasm and eventually to the nucleus, where it regulates downstream pathways. Activated PKD1 also regulates the process of vesicle trafficking from the Golgi to the membrane, which eventually controls cell surface proteins that are involved in cell adhesion, cell polarity, and motility. PKD1 has been shown to inhibit PCa. PKD1 inhibits tumorigenesis by enhancing cell adhesion and inhibiting the function of proteins involved in cell migration, cell invasion, cell proliferation, and EMT. PKD1 phosphorylates E-cadherin and β-catenin, thereby enhancing cell-cell adhesion. PKD1 helps to maintain cellular polarity by phosphorylating Par-1 polarity–associated kinase and thus enhancing its cytoplasmic sequestration by 14-3-3 protein. Activated PKD1 can also inhibit the transcriptional activity of β-catenin and AR, resulting in reduced cell proliferation. It also inhibits EMT by regulating the activity of snail transcription factor. PKD1 negatively regulates cell invasion by influencing the levels of MMPs through the modulation of HDACs. *AR* androgen receptor, *Hsp27* heat shock protein 27, *MMPs* matrix metalloproteins, *PKD1* protein kinase D1

believed to mediate this phosphorylation. Furthermore, overexpression of PKD1 suppresses the phosphorylation of c-Jun at Ser63 by EGFR which is a crucial in the regulation of proliferation and differentiation [200, 201].

Mounting evidence from our laboratory and others demonstrate that PKD1 plays an important role in PCa progression [202]. The down regulation of PKD1 in advanced prostate cancer was initially discovered in PCa cell line model by gene expression analysis [203]. The in vitro results were validated in several sets of human gene expression analysis. PKD1 is known to regulate membrane trafficking of proteins, cell adhesion and invasion [202]. Studies from our laboratory showed that PKD1 regulates the function of E-cadherin and β-catenin (cadherin-catenin complex) via interaction and phosphorylation. This regulation of cadherin-catenin complex by PKD1 enhances cell-cell attachment and suppresses cell motility and thereby inhibiting the invasion and metastasis of PCa [204, 205]. Additionally, PKD1 has been shown to decrease nuclear levels of β-catenin, one of the co-activators of AR-mediated transcription. PKD1 by decreasing levels of nuclear β-catenin affects the role of β-catenin, a transcriptional co-factor and thus attenuating oncogenic signaling pathways. Additionally, overexpression of PKD1 along with E-cadherin results in the decrease of cancer phenotype [206]. PKD1 also shown to modulate the functions of AR in prostate cancer cells [206–208]. Overexpression or knockdown models of PKD1 in cell lines revealed that PKD1 negatively regulates the function of AR [208] through the modulation of Hsp27-mediated AR functions [207]. Recently, we have identified that the PCa cell lines with low levels of PKD1 (C4-2 and E006AA cell lines) also have low levels of E-cadherin, and high levels of EMT markers like N-cadherin, snail, vimentin, MMP-2 and MMP-9 [209]. This suggests that PKD1 might play a pivotal role in suppressing levels of EMT markers. Altogether these data strongly support the critical role of PKD1 in prostate cancer.

PKD1 could be activated by pharmacological agents such as phorbol ester and Bryostatin 1 and by physiological stimuli such as platelet-derived growth factor, tumor-necrosis-factor, angiotensin II, and neuropeptide agonists [202, 207, 210, 211]. The decline in the cellular expression of PKD1 in advanced stage cancer has been reported to be due to epigenetic regulation [212]. Recently, strategies have been employed to selectively re-express PKD1 in breast cancer cell line using DNA methyltransferase inhibitor decitabine [213, 214]. Therefore, targeted upregulation of PDK1 expression and activity is a potential therapeutic approach in CRPC that could be explored.

Conclusion

Although androgen signaling has been most extensively studied and perhaps critical driver of PCa, it is not sole regulator of the disease and requires other bio-molecules and signaling pathways. An insight into the understanding of non-androgen signaling pathways may provide opportunities in development of novel drugs and therapies targeting CRPC. Targeting pathways beyond AR signaling have

potentials to further improve the treatment outcomes for patients. In fact, there are several drugs that are currently in clinical trials or being studied in preclinical and animal models. Most of these drugs act as specific inhibitors of dysregulated signaling pathways, such as those described in this chapter. Combination of strategies targeting non-AR signaling pathways along with the current therapeutic approaches remains promising in management of patient with metastatic PCa.

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Predictive Models in Castration Resistant Prostate Cancer

5

Tao Cui and Michael W. Kattan

Introduction

Median overall survival from clinical trials evaluating non-hormonal cytotoxic agents for patients with progressive metastatic prostate cancer following castration has gradually improved from 6 to 10 months [1] 30 years ago to 14–22 months [2–5] with recent trials reporting overall survival of over 32 months [6]. While it is debatable whether this improvement is strictly due to more efficacious therapies or from lead time bias due to early detection from isolated prostate-specific antigen (PSA) rise and improved imaging techniques, there has been a definitive decrease in the reluctance of physicians to administer and patients to accept these types of therapies. As the criterion for initiating chemotherapy following castration expands to include patients with PSA rise alone, the patient population will become increasingly heterogeneous, further increasing the important of differentiating individuals likely to experience good outcomes from those likely to experience poor outcomes. Tools that allow clinicians to stratify a patient's prognosis prior to initiating therapy are invaluable for not only counseling patients regarding their long-term outlook, but also to guide treatment algorithms to maximize on available therapeutic agents.

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A number of prognostic models have been designed retrospectively using datasets from various clinical trials. Individual models take into consideration various combinations of pre-therapeutic factors and parameters that indicate response during therapy. In addition, these models also utilize varying parameters, such as overall survival, time to bone metastasis, or a pattern of PSA increase, as predictive outcomes. Finally, each prognostic model often designed and validated using different datasets that may make them more or less applicable to specific patient groups. These factors taken together, can often lead clinicians to question which prognostic model to use, when to use it, and if it should be used at all. We seek to address these questions here as well as providing a brief overview of how prognostic models are constructed and validated, characteristics of reported models and how to compare them, and finally the advantages and limitations of prognostic models.

Basics of Predictive Modeling

Models are an essential tool for bridging known data with unknown or future outcomes. Models are able to condense much of what we observe about an individual patient into key parameters that affect the expectations of his present condition or future outcome. While for many years, the field of applied statistics has been dominated by the concepts of estimation and testing, prediction has become increasingly popular recently as an alternative philosophy [7]. The tenants of predictive modeling are not divergent from those of estimation modeling, but instead complement them by stressing different aspects of the same questions [8]. For example, when evaluating a dataset for factors that improve 5-year survival, a model based on estimation and testing would evaluate each parameter for significance and retaining only those factors that surpassed an established threshold. In contrast, a predictive model would value all parameters that improved the ability to predict outcomes for future patients. This raises two fundamental questions: "How can a predictive model be designed from a known dataset?" and "How can a predictive model be tested outside of the dataset from which it was created?".

Design and Validation of a Predictive Model

It should be noted at this point, that the usage of the term model is not necessarily limited to a presumed mathematic abstraction but rather to describe the way observed data can be represented in such a way that facilitates the predicting future outcomes. The goal of making accurate predictions is fundamentally agnostic to the mathematic model utilized in generating set. The primary concern of a robust predictive model is accuracy in producing predictions for future patients irrespective of the manner in which the model was constructed. Furthermore, if predictive accuracy is the most important characteristic, then the assumptions in any given model matter only to the extent that they affect accuracy. In other words, it is

preferable to include an unconventional assumption, if it improves the predictive accuracy. That being said, predictions are usually most accurate when obtained from sound models founded on plausible assumptions.

While it is philosophically simple to define the goal of a predictive model as accuracy in predicting future outcomes, it is practically difficult to measure this accuracy in when applied to future patients. At present, there is no ideal measure of predictive accuracy and this is an area of active and ongoing research [9]. However, there are methods that allow for the estimation of how well a model should perform in the future.

One of the most widely accepted methods is by using external datasets as a comparison. This requires a great deal of care because the predictive performance of a model on the dataset used to build it is biased and will exhibit over-fitting when compared to an independent data set. Therefore, the external dataset should be introduced as simulated future patients only after a model is fully developed and fixed to minimize contamination between the two groups.

Unfortunately, ideal external datasets, those derived from identical populations as the original dataset and receiving the identical therapies, are not always available. In these situations, an unmatched external dataset may underestimate the model's predictive accuracy and an alternative method must be used.

In the absence of external datasets, resampling of the original dataset can be used. Leave-one-out cross validation, also commonly called "jackknifing," is useful in these situations. With this methodology, each patient is individually omitted from the modeling process and later used as a test case. When each patient is omitted from the dataset, the remaining patients are used to construct a model that is then used to predict the outcome for the omitted patient. For example, with 10-fold cross validation, one tenth of the data are omitted from the modeling process, resulting in 10 models developed, each used to generate predictions for the tenth of patients omitted. At the end of the cross validation process, a data set is available that has a prediction for each patient that was based on a model that did not include that patient. In effect, this approach tries to replicate having an external data set. Cross validation is inferior to true external data set validation in at least two respects [10]: a truly external data set is a more stringent test of accuracy because the data are generated from a truly separate process; and cross validation is not testing a single model but a modeling approach because cross validation uses not a single prediction model but many (e.g., 1 per patient for jackknifing or 10 for 10-fold cross validation).

With a data set suitable for evaluating models, accuracy is commonly measured in 2 forms: discrimination and calibration [10]. Discrimination quantifies how well a model can rank patients with respect to their outcomes. Measures such as the concordance index or area under the receiver operating characteristic curve perform this. Calibration is usually a graphic assessment of model predictive accuracy, typically plotting predicted versus observed probabilities. These are the dominant approaches. However, once again, obtaining predictions is agnostic with respect to the actual measure of accuracy, and different analysts have their preferences and biases. New measures are an active area of research because no current measure is quantifiable, easily interpretable, and able to reflect truly calibration.

Choosing to Use a Predictive Model

Even though predictive models are constructed with the goal of predictive accuracy, not all models are constructed equally and no one model will fit all patients. With this in mind, how does a clinician judge which model to use? While selecting a model or ruling out a model based on the dataset from which it was derived seems like a reasonable first step, in actuality, the demographic composition of the derivative dataset may be of lesser concern than other factors.

Is the Treatment Delivered in the Model Dataset Incomparable to My Treatment?

Depending on the way the treatment modality changed over time, it may be possible to accommodate datasets that have discordant treatment types. For example, a gradual refinement in surgical technique can be adjusted for, such as by using an 'experience' variable, however, this may not be possible with dramatic changes such as the introduction of a new technology. Also, a completely new, and more effective, chemotherapy regimen would render an old model useless. The chemotherapy might be primary therapy, a neoadjuvant, adjuvant, or salvage therapy. If the old series used for developing the model lacked this impressive new regimen, the model would represent a worst-case scenario and probably not be useful for patient counselling. Similarly, radical changes in radiation therapy, such as a dramatically increased dose level, likely render an older model useless. In all of these cases, the new therapy should be thought to be radically more effective and not adjusted for in the model (e.g. not used at all in the derivation dataset).

Was the Period for Patient Accrual in the Model Dataset a Very Long Time Ago?

Diseases might change in their aggressiveness over time for reasons that are not explained by variables in the model. If the dataset is old, and particularly if the year of treatment was not included or examined as a predictor, this model might not be useful at all.

Do I Care About the Endpoint Predicted by This Model?

Some intermediate endpoints might be of little consequence and would exclude a nomogram from use.

Existing Models

Note on Memorial Sloan-Kettering Cancer Center (MSKCC) dataset: Several of the studies discussed below utilize all or part of an institutional dataset generated at MSKCC. This dataset consists of over 500 patients treated for metastatic prostate cancer from May 1989 to June 2000 using 19 treatment protocols, 15 of which were restricted to patients receiving castration therapy.

Emrich et al. [11]

Dataset: Derived from a dataset of with 1020 participants in clinical trials of the National Prostatic Cancer Project, of which, 605 having failed hormone ablation therapy
Treatment: Protocols were heterogeneous and included cyclophosphamide, 5-fluorouracil, estramustine phosphate, streptozotocin, imidazole-carboxamide, procarbazine, prednimustine, diethylstilbestrol, hydroxyurea, Methyl-chloroethyl-cyclohexynitrosourea, vincristine, methotrexate, and cis-platinum
Predictive Prognostic Factors (in order of decreasing importance): Hormone response, analgesic use, pain, elevated acid phosphatase, and anemia
Observed Outcomes: Objective response defined as progression, stable, partial response or complete response and survival time
Comments: Multivariate analysis on a large dataset, while the model fits the dataset well, no external dataset was used to validate the findings.

Fossa et al. [12]

Dataset: Derived from a clinical trial dataset of 58 patients
Treatment: Either flutamide or estramustine therapy
Predictive Prognostic Factors: PSA, hemoglobin, and fatigue
Predicted Outcomes: Survival at 4 versus 9 months
Comments: Subjects were subdivided into two groups with average survival times of 4 and 9 months respectively based on predictive prognostic factors, however, no external validation was performed.

Gravis et al. [13]

Dataset: Derived from the GETUG-15 study dataset of 385 patients
Treatment: Androgen deprivation therapy with or without docetaxel
Predictive Prognostic Factors: Alkaline phosphatase, Gleason score, and pain
Predicted Outcomes: Overall survival
Comments: 128 patients were withheld from the training analysis and used as a validation dataset. In the learning dataset, patients with normal alkaline phosphatase had median survival of 69.1 months versus 33.6 months for those with abnormal alkaline phosphatase.

Halabi et al. [14, 15]

Dataset: Two models derived from clinical trial datasets evaluating first (CALGB-90401) and second (TROPIC trial) line chemotherapy. CALGB-90401 trial consisted of 1050 patients and TROPIC trial consisted of 755 patients.
Treatment: Participants in the CALGB-90401 trial were randomly assigned to receive either docetaxel, prednisone, and placebo or docetaxel, prednisone, and bevacizumab. Participants in the TROPIC trial were randomly assigned to receive either 12 mg/m^2 of mitoxantrone plus 10 mg of oral prednisone daily or 25 mg/m^2 of cabazitaxel plus prednisone.
Predictive Prognostic Factors: ECOG performance status, lactate dehydrogenase, metastatic site, albumin, analgesic use, hemoglobin, alkaline phosphatase, and PSA
Predicted Outcomes: Overall survival
Comments: A subset of the subjects from each trial was withheld from the training analysis to be used as a validation dataset. In addition, external datasets from the ENTHUSE 33 trial and the SPARC trial were used in validation analyses.

Kelly et al. [16]

Dataset: Derived from MSKCC dataset using 110 patients with validation using an external dataset of 85 patients treated at a separate institution
Treatment: Protocols included of suramin, rhenium-186, estramustine, vinblastine, trimetrexate, and gemcitabine
Predictive Prognostic Factors: PSA decline of >50 %, lactate dehydrogenase
Observed Outcomes: Overall survival
Comments: Subjects were subdivided into low and high risk groups based on PSA response with median survival times of 8.6 months in the high risk group. Median survival time was not reached in the low risk group. External validation showed similar findings with low and high risk groups having median survival times of 10.8 and 8.5 months respectively.

Petrylak et al. [17]

Dataset: Derived from MSKCC dataset using 146 patients with cross validation and an external dataset of 29 patients
Treatment: Protocols included doxorubicin, methylgag, gallium, trimetrexate, etoposide, and difluromethylornithine. External validation dataset treated with suramin
Predictive Prognostic Factors: Lactate dehydrogenase, alkaline phosphatase
Observed Outcomes: Overall survival
Comments: Both Cox and exponential analyses were performed with congruent results regarding the significance of lactate dehydrogenase and alkaline phosphatase, however, Cox regression analyses fit the external validation dataset better.

Scher et al. [18]

Dataset: Derived from MSKCC dataset using 254 patients with validation using an external dataset of 541 patients from two randomized phase III trials
Treatment: Protocols included suramin, rhenium-186, bicalutamide, 13-*cis*-retinoic acid with IFN, edatrexate, and all *trans*-retinoic acid with liarozole, prednisone, and cyproterone
Predictive Prognostic Factors: PSA change, lactate dehydrogenase, hemoglobin, and age
Observed Outcomes: Overall survival
Comments: Median overall survival for patients with a >50 % decline in PSA levels was 23.6 months versus 12.3 months for those who did not show a post-therapy PSA decline. Validation of PSA decline with external dataset showed similar results with median survival of 20.8 versus 13.7 months respectively.

Smaletz et al. [19]

Dataset: Derived from an institutional dataset of 519 patients and validated using a randomized trial dataset consisting of 433 patients
Treatment: Protocols were variable consisting of 19 clinical protocols evaluating 15 different treatments. External validation dataset treated hydrocortisone plus suramin versus hydrocortisone alone
Predictive Prognostic Factors: Performance status, hemoglobin, lactate dehydrogenase
Observed Outcomes: Overall survival at 1 and 2 years
Comments: Results were validated by measuring the concordance index and calibration using a subset of the original dataset as well as by measuring against an external dataset.

Vollmer et al. [20, 21]

Dataset: Two hazard models derived from clinical trials by the Cancer and Leukemia Group B based on 137 patients in CALGB 9181 and 239 patients from CALGB 9182
Treatment: High or low dose megestrol acetate in the first dataset and low dose hydrocortisone alone or hydrocortisone plus mitoxantrone in the second dataset
Predictive Prognostic Factors: Both models identify PSA and PSA velocity as significant predictors. The second model also identifies hemoglobin and weight as significant predictors.
Observed Outcomes: Overall survival
Comments: The second model combines the datasets from both CALGB 9181 and CALGB 9182, however, neither model utilizes an external dataset for validation.

Ravi et al. [22]

Dataset: Validation of a model derived from the phase 3COU-AA-301 trial. Validation dataset consisted of 94 patients following treatment with docetaxel and 64 patients treated with abiraterone but pre-docetaxel treatment.
Treatment: Either docetaxel or abiraterone before docetaxel
Predictive Prognostic Factors: ECOG performance status, liver metastases, duration of androgen deprivation therapy, albumin, alkaline phosphatase, and lactate dehydrogenase
Predicted Outcomes: Overall survival
Comments: Application of the risk stratification model was able to prognosticate overall survival (hazard ratio good vs. intermediate: 2.73 [95 % confidence interval [CI], 1.61–4.64], good vs. poor: 3.79 [95 % CI, 1.52–9.45]).

Templeton et al. [23]

Dataset: Derived from an institutional dataset of 357 patients. A validation dataset was used consisting of 215 patients treated at a separate institution.
Treatment: Docetaxel
Predictive Prognostic Factors: ECOG performance status, hemoglobin, alkaline phosphatase, PSA, and neutrophil-to-lymphocyte ratio
Predicted Outcomes: Overall survival at 1, 2, and 3 years
Comments: Four risk categories were identified based on the number of poor prognostic factors. Overall survival at two years was 43 % for individuals with 0 poor prognostic factors and 3 % for those with 3–5 poor prognostic factors.

Choosing Between Reported Models

Where there are multiple predictive models that could apply to a given situation, here are some considerations for evaluating which one to use.

1. *Equation > nomogram > risk groups > single Kaplan-Meier curve*: A mathematical equation is probably going to be the most accurate prediction method available. For all practical purposes, this has to be in software. The next best tool is a nomogram, etc.
2. *Regression models are better than classification and regression tree (CART) models*. Again, this is 'all else being equal' for each of these criteria, but rarely will a CART predict more accurately than a properly constructed regression equation.
3. *Greater sample size is better*. The model made from the larger dataset would be preferred, all else being equal.
4. *More predictors in the model is better*. With the emphasis on addressing accuracy, not practicality, the model that includes all routinely available predictors that are thought to be predictive/prognostic is usually more accurate.

5. *No variable selection is better than variable selection (based on P values)*. Stepwise variable selection methods tend to produce less accurate prediction models. Models that contain only those variables that were statistically significant in univariable analysis are also going to be inferior, in general, to full models that have no univariable screening. In short, I favor the model that ignored P values and model fit statistics throughout the entire model building process.
6. '*Continuous variables that are kept continuous*' *is better*. Constraining a continuous variable to a categorical variable may reduce predictive accuracy, albeit slightly.
7. *Continuous variables allowed to have nonlinear effects (e.g. with splines) is better*. A model that relaxed the linearity assumption, or at least examined the linearity assumption, is going to be better than one did not, typically.
8. *Fewer missing values is better*. Many missing values in the dataset from which the model was developed tends to suggest systematic issues with the data. Methods such as imputation are limited as a remedy.
9. *A higher concordance index is better*. This is difficult when the models are not compared directly on neutral data. The model that achieved the greater concordance index (which needs to be properly corrected for over-fit to be meaningful) may be more accurate though, the difference may be insignificant.
10. *Closer to the 45° calibration curve is better*. Sometimes this is difficult to judge, because the calibration lines tend to vacillate.

Advantages of Nomograms

Nomograms are a particular type of predictive modeling that condenses the effect of individual prognostic factors into a tailored predicted probability. As a result, nomograms are able to calculate personalized risk on an individual patient basis and have been shown to be more accurate than using risk group strategies [24, 25]. In fact, nomograms have been shown to outperform clinicians as predictors of future outcomes in a number of areas [26, 27] including prostate cancer [28, 29]. While able to delivering more accurate predictions, nomograms are also more complex than risk group strategies and this, at one time, limited their usefulness. However, the modern ubiquity of computer resources has greatly increased the approachability and utility of digital nomograms. Ultimately, because nomograms simultaneously consider multiple aspects of the patient's cancer (stage, grade, PSA level, etc.), a more accurate prediction for an individual patient is obtained.

Limitations of Nomograms

The main limitation to the predictive accuracy of a nomogram is the quality of the dataset on which it was based. As discussed above, if the treatment protocol or prognostic factors used to derive a nomogram differ significantly from those of the

patient, then the predictive accuracy of that nomogram may be significantly impaired. In addition, nomograms are specific for a given outcome, and the most important outcomes, often cancer specific death and health-related quality of life in the case of prostate cancer, are also the most difficult to predict. Cancer specific death is a relatively rare event that takes many years to observe and health-related quality of life is subjective and difficult to measure on a regular basis. Both of these factors make datasets containing these outcomes both rare and costly to generate. Most importantly, the nomogram is meant as a supplement and not a replacement for the decision making process. Nomograms do not consider the patient's perspective on their disease, the impact of treatment complications or the goals of therapy. If these variables are clearly defined between the clinician and patient, then nomograms are the most accurate predictive models currently available and provide valuable risk assessments for a variety of clinical endpoints.

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Docetaxel in Advanced and Castration Resistant Prostate Cancer

6

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Introduction

Cytotoxic chemotherapy for castration resistant prostate cancer was once considered to be toxic and ineffective. Initial results with cytotoxic agents demonstrated objective response rates of 6.5–8.7 % in men with metastatic castrate resistant prostate cancer, with palliative improvement without a survival benefit [1, 2]. In 2004, two randomized trials demonstrated that docetaxel based therapy had superior survival in castrate resistant metastatic disease when compared to the then standard of care, mitoxantrone combined with corticosteroids. Recent studies demonstrating a survival benefit for docetaxel in advanced castrate sensitive disease have expanded its use in this setting as well. This chapter will summarize the data of docetaxel in patients with metastatic disease and briefly review the data of its use in local disease.

Mechanisms of Docetaxel in Prostate Cancer

Docetaxel (chemical formula, $C_{43}H_{53}NO_{14}$ and M.W. 807.9 g mol^{-1}) is a water insoluble anti-mitotic chemotherapeutic agent in the taxane family [3, 4]. The taxanes include docetaxel (Taxotere), cabazitaxel (Jevtana), and paclitaxel (Taxol)

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and exert their cytotoxic effects through a number of mechanisms described below but the most well-described is through microtubule stabilization. Due to its hydrophobic nature, docetaxel penetration into cancer cells requires the use of plasma proteins such as lipoproteins, albumin and α1 acid glycoprotein [3]. Hydroxylation of the *tert*-butyl group at the C_{13} side chain occurs intracellularly, leading to a cyclical formation which binds to microtubules [5]. Docetaxel is actively cleared from cells through efflux by members of the ATP-binding cassette (ABC) transporter family, metabolized intracellularly by drug detoxifying proteins, such as glutathione-S-transferase P1, and metabolized in the liver by the cytochrome P450 system, with particular activity from CYP3A4 [3, 6–8].

Microtubules are a key cellular structural component in all cells, necessary for intracellular translocation and mitotic spindle formation. They are comprised of heterodimers of alpha and beta tubulin. Docetaxel binds to the B-tubulin component, inhibiting two key dynamic behaviors of microtubules—dynamic instability and treadmilling [9]. This interrupts mitosis by disrupting mitotic spindle formation and leading to G(2)M phase cell cycle arrest [3, 10]. Beyond cell cycle arrest, disruption of microtubule dynamics has been implicated in at least two other areas of cellular biology of importance in prostate cancer; by inducing phosphorylation of bcl-2 and through the disruption of androgen receptor (AR) translocation to the nucleus. Bcl-2 is an antiapoptotic protein which has been shown by immunohistochemistry to be increased in androgen-independent metastatic prostate cancer cells. Docetaxel induces its phosphorylation, leading to direct activation of pro-apoptotic cascades [10–12]. The AR is a ligand-dependent nuclear transcription factor with a well-established role in prostate cancer, known to have persistent activity in castrate resistant disease [13]. Docetaxel and other taxanes have been shown to disrupt AR translocation to the nucleus thereby interfering with downstream signaling and transcriptional activation [14, 15]. However, a recent in vitro study of docetaxel (and cabazitaxel) at clinically attainable nanomolar concentrations found no impact on AR localization despite levels sufficient to exert cytostatic and cytotoxic effects, challenging our understanding of the role of docetaxel in AR activity [16].

Docetaxel in Metastatic Castrate Resistant Disease

Early phase I and II studies of docetaxel as a single agent or in combination with estramustine established the safety of the drug and showed promising activity with PSA decline rates of >50 % ranging between 36–69 % of treated men with metastatic castrate resistant prostate cancer (mCRPC), objective response rates between 17–38 % and median survivals between 9–23 months [17–22]. These studies paved the way for two separate phase III studies examining docetaxel in mCRPC, SWOG 99-16 and TAX 327, which showed improved overall survival in their docetaxel treatment arms, leading to FDA approval of the drug in this setting.

SWOG 99-16 randomized 770 men with mCRPC to one of two 21-day treatment cycles with either docetaxel 60 mg/m2 on day 2 plus estramustine 280 mg on days 1–5 plus dexamethasone 60 mg in three divided doses prior to docetaxel, or

mitoxantrone 12 mg/m2 plus prednisone 5 mg BID. The study showed a significant improvement in the primary endpoint of OS in the docetaxel plus estramustine group compared to the mitoxantrone group (17.5 months vs. 15.6 months). Furthermore, two of three secondary endpoints showed significant results favoring the docetaxel plus estramustine group, with a median time to progression of 6.3 months versus 3.2 months compared to the mitoxantrone group ($P < 0.001$) and PSA response (defined as post-treatment declines of at least 50 %) in 50 % of patients in the docetaxel plus estramustine group compared to 27 % in the mitoxantrone group ($P < 0.001$). A third secondary endpoint of overall response was observed in 17 % of patients receiving docetaxel plus estramustine versus 11 % of patients receiving mitoxantrone, although this result did not achieve statistical significance ($p = 0.30$) [23].

Mitoxantrone had been the standard of care in mCRPC based on earlier studies showing improvement in bone pain and time to progression, despite failing to demonstrate an improvement in survival [24, 25]. Estramustine was used in the treatment arm with docetaxel due to early preclinical and clinical studies suggesting synergy with docetaxel, felt to be due to its disruption of microtubule-associated proteins [20, 26]. However, in a subsequent study of 150 mCRPC patients randomized to docetaxel with or without estramustine, the addition of estramustine did not achieve a statistically significant increase in PSA response (again measured as a decrease in PSA of 50 %). Additionally, there was significantly more grade 3 or 4 toxicity as well as a non-significant decrease in OS (19.3 months vs. 21 months) in the docetaxel plus estramustine group compared to the docetaxel only group [27].

TAX 327 randomized 1006 men with mCRPC to one of three therapies: mitoxantrone 12 mg/m2 every 3 weeks, docetaxel 75 mg/m2 every 3 weeks, or weekly docetaxel 30 mg/m2 for 5 of every 6 weeks; all groups received prednisone 5 mg BID concurrently. The primary end point was overall survival and secondary end points were pain, PSA levels, and quality of life. Patients in the q3 week docetaxel group had a hazard ratio for death of 0.76 compared to those in the mitoxantrone group (95 % confidence interval, 0.62–0.94; $P = 0.009$) and a median survival of 18.9 months compared to 16.5 in the mitoxantrone group. The survival improvement in the weekly docetaxel group compared to the mitoxantrone group did not reach statistical significance. Patients receiving weekly docetaxel or those receiving docetaxel every 3 weeks both had significant decreases in pain, significantly more PSA declines of >50 %, and statistically significant improvement in quality of life compared to those receiving mitoxantrone [28].

Due to a subset of patients with initial rises in PSA with subsequent declines in the TAX 327 cohort, it has become accepted that early increases in serum PSA (up to 12 weeks) should be ignored when determining response or progression. The study also noted median survival for minimally symptomatic patients was 28.4, 25.9, and 22.0 months for the q3 week docetaxel, weekly docetaxel, and mitoxantrone groups, respectively. Although these differences were not statistically significant due to small numbers of men with minimal symptoms in each of these groups, this raised the suggestion for a role for docetaxel in earlier, less advanced disease.

Subsequent analysis of both the SWOG 99-16 and TAX 327 data revealed that PSA response was significantly associated with overall survival. In the SWOG 99-16 cohort, a 3 months PSA decline of at least 30 % was associated with a more than 50 % decrease in the risk of death compared with the lack of such a decline (HR 0.43, 95 % CI = 0.34–0.55; $P < 0.001$) and in the TAX 327 cohort, men with PSA response (as defined in the initial study as a decline of at least 50 %) lived significantly longer than men without PSA response (HR 0.45; 95 % CI = 0.39–0.53; $P < 0.001$) [29, 30]. Although surrogacy for overall survival has not been fully accepted, PSA response nonetheless has become accepted as an important endpoint in the treatment of prostate cancer.

The optimal duration of docetaxel in mCRPC remains unclear. The FDA label, which recommends 10 cycles of docetaxel, is based on the fact that both TAX 327 and SWOG 99-16 capped the number of docetaxel cycles in each arm at 10, to be comparable to the mitoxantrone dose. Thus, the optimal number of cycles of docetaxel, balancing efficacy and toxicity, has yet to be defined. A retrospective analysis of the TAX 327 study and a phase II study of docetaxel with or without the bcl-2 inhibitor AT-101, which allowed up to 17 cycles, showed a trend toward inferior survival in men with mCRPC receiving fewer than 10 cycles of docetaxel for reasons other than disease progression or death, and also showed continued PSA declines with up to 17 cycles [31, 32]. Despite the PSA declines, a survival benefit was not detected with more than 10 cycles. Additionally, intermittent docetaxel therapy has been shown to result in repeat PSA responses and data from the ongoing PON-PC-O2 trial of androgen withdrawal and intermittent versus continuous docetaxel may help clarify the utility of this approach [33, 34].

Resistance to Docetaxel

Docetaxel resistance is complex and incompletely understood. A number of mechanisms have been implicated in the development of taxane resistance including increased drug efflux, impaired microtubule binding, interactions with microtubule-associated proteins, defects or mutations in mitotic checkpoint signaling, induction of epithelial-to-mesenchymal transition, upregulation of signaling pathways controlling stem-cell renewal and cell differentiation, and challenges in tissue penetration and drug delivery [35].

Increased drug efflux from cancer cells is one well described mediator of docetaxel resistance, resulting from drug binding to proteins of the ATP-binding cassette (ABC) transporter family. For example, ABCB1 (also called MDR1 and P-glycoprotein 1), ABCC4 and ABCB5, have all been shown to be involved, and their respective inhibition in vitro has been shown to reverse docetaxel resistance [36–38]. Decreased binding of the drug to microtubules may occur both through mutations in tubulin genes as well as overexpression of specific tubulin isoforms. Overexpression of the β-tubulin III isoform has been shown to predict docetaxel resistance, felt to be due to decreased binding of the drug to that isoform [39]. Although not the primary site of docetaxel binding, mutations in α-tubulin have

also been shown to confer resistance to the drug through changes in microtubule associated protein binding leading to elevated levels of microtubule destabilizing factors and altered microtubule dynamics [40]. Various splice variants of the AR are one mechanism by which prostate cancer cells may develop resistance to both castration as well as docetaxel. Recent work has shown that tumor xenografts containing different AR splice variants have differing sensitivity to docetaxel possibly due to loss or changes of the microtubule binding domain of AR [41]. At the same time, docetaxel may play a particularly important role in men with specific AR splice variants, such as AR-V7, which has been shown to confer resistance to enzalutamide and abiraterone, as men with AR-V7 have significantly greater PSA responses and PSA PFS when treated with docetaxel compared to enzalutamide or abiraterone [42, 43].

Epithelial-to-mesenchymal transition (EMT) and the overexpression of signaling pathways involved in stem-cell self-renewal and embryonic differentiation such as Notch and Hedgehog promote invasiveness and migration of cancer cells and lead to cells with stem-cell-like properties and drug resistance [44–46]. Zinc finger E-box binding homeobox 1 (ZEB1) is a transcription factor that promotes EMT and its role in taxane resistance has been shown in docetaxel resistant cell lines by reestablishing docetaxel sensitivity after transfection of ZEB1 siRNA [47, 48]. Docetaxel resistant cell lines have been shown to have decreased expression of epithelial cell markers CK18 and CK19 and HLAI antigens as well as increased activity of the Notch and Hedgehog pathways. Cells with this drug-resistant phenotype are found in even early prostate cancer tissue samples and become increasingly abundant in both metastatic tumors and after docetaxel exposure, and inhibition of Notch and Hedgehog pathways in vivo results in the re-establishment of docetaxel activity in prostate cancer cell lines [49].

Findings from other taxanes may help explain docetaxel resistance as well. Overexpression of microtubule-associated destabilizing proteins such as stathmin and inactivation via phosphorylation of microtubule associated stabilizing proteins such as MAP4 have been shown to decrease sensitivity to paclitaxel in vitro [35]. Mitotic checkpoint signaling is one system by which cells prevent the progression of the cell cycle from mitosis to anaphase [50]. Reduced expression of checkpoint genes has been shown to confer reduced sensitivity to paclitaxel, possibly by counteracting the G(2)M arrest induced by taxanes [35].

Drug delivery to target tissues is a concern with any drug, but limited tissue penetration of taxanes in particular has been described as another mechanism of tumor resistance to docetaxel, leading to the call for alternate tissue delivery systems such as nanotechnology [4, 51]. Nanoparticle systems of drug delivery such as liposomal encapsulation or polymeric encapsulation of chemotherapeutic drugs have been proposed to increase drug solubility, protect from drug degradation, and help overcome efflux by P-glycoproteins. There is currently an ongoing phase II study of a prostate specific membrane antigen (PSMA) targeted polymeric nanoparticle containing docetaxel [52–54].

The role of docetaxel rechallenge in the sequencing of castration resistant prostate cancer treatment has yet to be defined. However, in settings where docetaxel was discontinued for reasons other than disease progression or toxicity, retreatment with docetaxel after progression has been shown to result in further PSA responses in both retrospective studies and a prospective phase II study [55–57].

Docetaxel versus Cabazitaxel

The only other chemotherapeutic shown to improve survival in mCRPC is cabazitaxel, a semisynthetic taxane. The phase III TROPIC clinical trial (NCT00417079) established the survival benefit for cabazitaxel compared to mitoxantrone in patients who had already progressed on docetaxel, with an overall survival of 15.1 months in the cabazitaxel group versus 12.7 months in the mitoxantrone group (HR 0.7, $p < 0.0001$) [58]. Several mechanisms have been proposed to account for the efficacy of cabazitaxel after progression on docetaxel. Cabazitaxel was developed in part due to its decreased affinity for the efflux protein glycoprotein 1 [59]. In addition to decreased efflux from cells, cabazitaxel and docetaxel seem to exert their cytotoxic effects through differing molecular pathways. In vitro gene-expression analyses of both castrate sensitive and castrate resistant cell lines have shown distinct genomic responses of cabazitaxel from docetaxel, with cabazitaxel showing more significant effects on cell-cycle and chromatin regulation genes and docetaxel showing stronger impact on transcription and cell repair mechanisms [16]. Trials are now comparing docetaxel to cabazitaxel as front line therapy (NCT01308567) [60].

Docetaxel in Combination with Other Therapies

While in vitro evidence suggests that antiangiogenesis agents, bone targeting agents, and vaccine agents may synergize with docetaxel, no phase III trial to date combining targeted agents with docetaxel in mCRPC has reported a survival benefit compared to taxane monotherapy. This failure may be in part due to the relative lack of efficacy of some of these agents as monotherapy, patient selection in phase I/II studies, as well as the heterogeneity of the disease. The ASCENT trial randomized patients with mCRPC to weekly docetaxel with calcitriol versus every 3 week docetaxel based on earlier data showing tolerability, PSA response rates, and an adjusted survival benefit in patients treated with this combination [61]. However the combination arm ended up having shorter survival than the q3 week docetaxel control arm [62]. The effect of the weekly dosing strategy on this result is unclear. CALGB 90401 compared docetaxel and prednisone with or without bevacizumab. While the combination arm did show an improvement in progression free survival and overall response, it did not improve overall survival and was associated with greater toxicity [63]. Another VEGF inhibitor, aflibercept, was tested in combination with docetaxel in the VENICE trial, and also showed greater

toxicity and failed to show an improvement in overall survival compared to docetaxel with placebo [64]. Results of the MAINSAIL trial examining docetaxel with or without the immunomodulatory agent lenalidomide, which also has antiangiogenic properties, showed a decrease in overall survival in patients receiving the combination compared to those receiving docetaxel alone [65].

Despite the propensity of prostate cancer to metastasize to bones, results of phase III trials combining docetaxel with bone targeted agents have also been largely negative. Prostate cancer cells increase osteoblast activity and decrease osteoclast activity via stimulation of ET-1, which exerts its actions via the endothelin receptor [66, 67]. In the ENTHUSE M1C trial, the addition of the oral endothelin receptor antagonist zibotentan to q3 week docetaxel did not significantly improve overall survival compared to docetaxel plus placebo [68]. SWOG S0421 tested another endothelin receptor antagonist atrasentan in combination with docetaxel, but again the combination did not improve overall survival or PFS compared to docetaxel alone [69]. The tyrosine kinase inhibitor dasatinib was felt to possibly have a role in mCRPC via inhibition of src kinase-mediated promotion of bone metastases, but similar to the endothelin receptor antagonists, the addition of dasatinib to docetaxel in the READY trial did not improve overall survival compared to docetaxel alone [70].

A phase II study of the ProstVac VF vaccine in combination with docetaxel showed that the addition of chemotherapy would not inhibit T-cell responses and hinted that patients with mCRPC treated with vaccine may respond longer to docetaxel with PFS of 6.1 months compared to an historical docetaxel only control of 3.7 months [71]. Unfortunately the VITAL-2 phase III study comparing the whole-tumor cell vaccine GVAX with or without docetaxel in patients with symptomatic mCRPC was stopped early due to increased deaths in the combination group and analysis of the prematurely closed study showed decreased median survival in the combination arm compared to docetaxel only (12.2 vs. 14.1 months) [72].

Other drug combinations with docetaxel, including targeted agents, hormonal agents, and chemotherapeutics, have shown some promise in phase II studies or are currently under investigation. Results from a phase I/IIa study of the bone targeted agent radium-223 showed increases in PSA responses >50 % (61 % vs. 54 %) and normalization of bone alkaline phosphatase (91 % vs. 64 %) in patients receiving the combination of radium-223 with docetaxel compared to docetaxel alone [73]. Of concern is the fact that both docetaxel and radium-223 had their doses or schedules modified. Clusterin is an antiapoptotic chaperone protein that is overexpressed in docetaxel-refractory cell lines. Knockdown of clusterin with custirsen, an antisense inhibitor of clusterin, has been shown to resensitize docetaxel refractory prostate cancer cells to docetaxel as well as other cytotoxic chemotherapies [74]. While the phase III SYNERGY trial failed to show a survival benefit with the addition of custirsen to docetaxel in the study population of treatment naïve men with mCRPC, subgroup analysis did show improvement in median overall survival with docetaxel plus custirsen compared to docetaxel alone in poor-prognosis

patients (17.0 months vs. 14.0 months, stratified HR = 0.72, P = 0.0026) [75]. Other antiapoptotic agents have also failed to show benefit in phase II studies. Bcl-2 is an antiapoptotic protein that is highly expressed in many cancers, including castrate resistant prostate cancer cells [76]. A randomized phase II study of the antisense oligonucleotide oblimersin in combination with docetaxel showed increased toxicity and failed to show benefit compared to docetaxel alone [77]. Similarly, another phase II study of an alternate inhibitor of bcl-2, AT-101, also failed to show benefit when combined with docetaxel [31].

Given the established benefit of antiandrogens in prostate cancer, hormonal agents have also been tested with docetaxel in phase II studies. Orteronel is a non-steroidal, selective inhibitor of the 1720-lyase activity of CYP17A1, thereby suppressing the conversion of androgen precursors to androgens. Results from a phase I/II study of docetaxel in combination with orteronel showed promise for this combination with evidence for PSA and radiologic responses with this combination [78]. CHEIRON is a phase II study currently underway investigating the addition of the antiandrogen enzalutamide to docetaxel in the first line setting for mCRPC (NCT02453009) [79].

Cabazitaxel is the only other cytotoxic chemotherapy with an established survival benefit in mCRPC. However, several other chemotherapeutic agents have been tested in combination with docetaxel. As previously reviewed, the addition of estramustine to docetaxel led to increased toxicity without survival benefit [27]. The addition of cyclophosphamide to docetaxel showed no additional benefit to cyclophosphamide in a phase II study [80]. Several studies have examined the addition of platinum agents to docetaxel, both with and without estramustine, in mCRPC with promising response rates even after progression on docetaxel. To date, however, no studies have shown a survival benefit with the addition of platinum therapy [81–83].

Docetaxel in Metastatic Castrate Sensitive Prostate Cancer

Given the established survival benefit of taxanes in advanced mCRPC, three separate trials aimed to assess the benefit in metastatic castrate sensitive disease. These include the CHAARTED trial, the STAMPEDE trial, and the French GETUG-AFU 15 trial. The CHAARTED trial (ChemoHormonal Therapy versus Androgen Ablation Randomized Trial for Extensive Disease in Prostate Cancer; ECOG3805) randomized 790 men with newly diagnosed metastatic prostate cancers to treatment with either androgen deprivation therapy (ADT) or ADT plus 6 cycles of q3 week docetaxel (without the addition of prednisone). Patients were stratified prospectively into cohorts reflecting “low” versus “high” burden of disease, with high volume disease including those patients with >4 bony metastases, any metastases outside of the vertebral column or pelvis, or extranodal visceral metastases. Among the overall study population, overall survival and time to progression were

significantly improved in the docetaxel plus ADT arm compared to the ADT only arm (OS 57.6 months vs. 44.0 months, P < 0.001; median time to progression 20.2 months vs. 11.7, P < 0.001). The improvement in OS was particularly dramatic among patients with "high volume" metastatic disease (49.2 vs. 32.2 months, p = 0.0006). Of note, at the time of publication, median OS had not yet been reached for either arm of the low-volume group [84].

The French GETUG-AFU 15 randomized 385 men with newly diagnosed castrate sensitive metastatic prostate cancer to either ADT alone or ADT with up to 9 cycles of docetaxel. Despite significant improvement in biochemical (23 months vs. 13 months) and clinical progression free survival (23 months vs. 15 months) in favor of the docetaxel arm, three year OS was essentially the same between the two groups (64.2 % in the ADT plus docetaxel group versus 62.9 % in the ADT alone group). Median OS at 50 months was 58.9 months in the ADT plus docetaxel group versus 54.2 months in the ADT alone group, although this difference was not statistically significant. The researchers also reported 72 serious adverse events (including four treatment-related deaths), in the ADT plus docetaxel group compared to no serious adverse effects in the ADT alone group, and thus concluded that docetaxel should not be used in first line therapy of non-castrate resistant metastatic prostate cancer [85]. This trial was approximately half the size of the CHAARTED study, which may have resulted in under powering.

The STAMPEDE trial (Systemic Therapy in Advanced or Metastatic Prostate Cancer: Evaluation of Drug Efficacy; NCT00268476) is a multi-arm trial which includes an arm of 1087 men with mCSPC randomized to either ADT alone or ADT with 6 cycles of docetaxel. Overall survival for the ADT plus docetaxel group was significantly improved with a HR of 0.73 [86]. Of note, no stratification based on disease volume has been performed. The CHAARTED and STAMPEDE studies have resulted in the standard use of docetaxel with ADT in mCSPC in those patients with high volume disease. The role for docetaxel in lower volume disease remains unclear, and may be strengthened with further maturation of this cohort in the CHAARTED study.

Docetaxel in Local Disease

While docetaxel is firmly established in the management of metastatic prostate cancer in both increasing survival as well as palliation of symptoms, the role for docetaxel in potentially curative therapy (neoadjuvant or adjuvant) of high risk local disease remains unclear. There have been a number of phase II trials of neoadjuvant docetaxel both with and without ADT prior to radical prostatectomy. While there has been some evidence of biologic activity with reported decreases in pre-surgical PSA levels and down staging in both neoadjuvant chemotherapy and chemohormonal trials, the clinical benefit of this effect remains uncertain, as none of the phase II trials have shown improved clinical outcomes [87]. Furthermore, no

patients in the neoadjuvant docetaxel trials without ADT achieved a pathologic complete response (pCR). With reported pCR rates between 0–11 %, the phase II neoadjuvant chemohormonal trials to date have not been encouraging either [87]. While pCR has been shown to be associated with improved survival in both breast and bladder cancers, its clinical association in prostate cancer remains unclear [88, 89]. There is an ongoing randomized phase III trial (NCT00430183) comparing docetaxel + ADT to no therapy prior to prostatectomy in high-risk localized disease that has completed accrual and should help to clarify the utility of neoadjuvant docetaxel [90].

Trials of adjuvant hormonal therapy in high risk localized prostate cancer patients have been plagued with difficulty due to lack of consensus in patient selection criteria and the lack of validated measurable intermediate endpoints, necessitating long-term follow up, and few investigators have attempted adjuvant trials with chemotherapy. To date there have been two phase III studies examining the role of docetaxel in the adjuvant setting. TAX 3501 was a four arm multinational phase III study designed to compare adjuvant ADT with or without docetaxel administered either immediately after radical prostatectomy or at the time of subsequent PSA progression. Unfortunately the study was closed early due to poor accrual [91]. RTOG 0521 is a multi-institutional phase III study which randomized 563 patients with high risk localized prostate cancer (1—Gleason 7–8, any T-stage, and PSA > 20, or 2—Gleason 8, ≥T2, any PSA, or 3—Gleason 9–10, any T-stage, any PSA) to receive ADT for 24 months with external-beam radiation therapy with 75.6 Gy over 8 weeks, with our without 6 cycles of q3 week docetaxel 75 mg/m2 starting 4 weeks after the completion of radiotherapy. At a median-follow up of 5.5 years, the 4-year overall survival rate was 89 % for men who received ADT and RT versus 93 % for men treated with ADT, RT, and docetaxel, representing a 32 % relative reduction in the risk of death for patients randomized to the docetaxel arm (HR = 0.68; 95 % CI, 0.44–1.03; P = 0.03) [92].

GETUG 12 is a French phase III study that examined the role of docetaxel in the management of local disease but not within traditional neoadjuvant or adjuvant parameters. Treatment-naïve prostate cancer patients underwent pelvic lymph node dissection and those with one or more high risk feature (stage >= T3, Gleason ≥ 8, PSA > 20 ng/mL, or pathological node-positive disease) were randomized to receive 3 years of ADT with or without the addition of four cycles of q3 week docetaxel 70 mg/m2 on day 2 and estramustine 10 mg/kg per day on days 1–5. Patients in both cohorts could go on to receive local therapy at 3 months after the start of treatment. Local therapy was decided upon by multidisciplinary tumor boards and consisted of either radiotherapy or radical prostatectomy in the case of node negative disease or radiotherapy or no local treatment in the case of node positive disease. The 8-year relapse-free survival was 62 % in the ADT plus docetaxel and estramustine group versus 50 % in the ADT only group (HR = 0.71, 95 % CI 0.54–0.94, p = 0.017), again representing a nearly 30 % reduction in the relative risk of recurrence or death in the chemotherapy arm [93].

Conclusions

Docetaxel, along with cabazitaxel, remain the primary and secondary chemotherapeutic agents in advanced castrate resistant prostate cancer. Findings in castrate sensitive disease have broadened the use of docetaxel and may mark the beginning of a trend of evaluating these agents in earlier disease. Recent findings showing clinical benefit in high risk local disease, which need further confirmation as well as ongoing trials of chemohormonal and combination therapy with novel agents may continue to expand the role for docetaxel in the coming years.

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Combination Treatment Strategies with Docetaxel in Patients with Metastatic Prostate

7

Ben Fulton and Robert J. Jones

Prior to the development of the taxanes, cytotoxic chemotherapy was considered relatively ineffective in the management of men with metastatic castrate-resistant prostate cancer (mCRPC). Early clinical trials demonstrated objective responses in 10–20 % of men and median survival rarely exceeded 12 months.

TAX327 changed this. This phase III trial randomly assigned 1006 men with chemotherapy-naïve mCRPC to receive either docetaxel (75 mg/m^2 every 3 weeks), docetaxel (30 mg/m^2 weekly) or Mitoxantrone (12 mg/m^2 every three weeks). All patients received prednisone 5 mg orally twice per day. The trial demonstrated improvement in its primary endpoint of overall survival for 3-weekly docetaxel when compared to mitoxantrone (19.2 vs. 16.3 months respectively, p = 0.004) although it failed to show overall survival gain within the weekly docetaxel group (median survival 17.8 months, p = 0.09). The 3-weekly regimen of docetaxel was also associated with higher PSA response rates than mitoxantrone (45 vs. 32 %). Grade 3 or 4 neutropenia was more common with docetaxel given every 3 weeks compared to either weekly docetaxel or mitoxantrone regimes (32 vs. 2 vs. 22 % respectively) [1, 2].

These and other results established docetaxel/prednisone combination therapy as the standard of care for men with mCRPC and increased interest throughout the

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world in the use of other cytotoxic regimens and their therapeutic role in this setting. As the new standard of care, docetaxel also became a new control for future drug development.

Why Do Combination Studies with Docetaxel in Prostate Cancer?

The development of effective new drugs for the treatment of advanced prostate cancer has, at least until recently, lagged significantly behind other common solid tumors. Docetaxel was the first drug to conclusively demonstrate improvement in overall survival in castration resistant disease and so its use rapidly became a new paradigm in treatment. There are multiple drivers in the pathways of drug development and these pathways had to encompass docetaxel as a new standard of care.

Biology and Combination Therapy

One consideration was the drive to find drugs that were truly synergistic with docetaxel whereby the combination of the two drugs would result in greater efficacy than the sum of its parts if given in sequence. The mechanisms for synergy vary, including enhanced cytotoxicity or inhibition of drug resistance mechanisms.

Complementary Toxicity

One also needs to consider the likely toxicities of combination therapy, ideally combining drugs with complimentary, rather than similar toxicity profiles. By and large there is reticence in combining drugs where the dose of either drug is compromised, especially if there is a risk of reducing the dose intensity of the standard treatment.

Ethical Considerations

Where there is an accepted standard of care, such as is the case with docetaxel in mCRPC, then it is likely that a clinical trial in which this standard is either denied or delayed may be considered unacceptable to patients and ethics committees, especially where prior clinical efficacy data for the novel therapy are few. Thus, randomized head-to-head trials comparing new drugs with docetaxel in the control arm only may prove challenging. However, randomized trials where all patients receive docetaxel may be perceived as more acceptable on the basis that no patient is denied timely docetaxel, even thought there is a risk that delivery or efficacy may still be compromised by combination with the novel drug.

Clinical Considerations

Until the availability of cabazitaxel, there was no proven life-prolonging therapy suitable for men who had previously failed docetaxel. Indeed several new drugs failed to demonstrate efficacy in this group [3, 4]. By and large patients who had failed docetaxel in this era had a relatively poor prognosis and a high burden of morbidity and, often, co-morbidity making this a relatively unattractive population in which to develop new drugs. Subsequent successes in the post chemotherapy setting, including abiraterone, enzalutamide, cabazitaxel and radium-223 have clearly demonstrated that it is possible to succeed in this niche but, nonetheless, combination with docetaxel ensures a population of patients who are clearly fit for systemic therapy and who are not heavily pretreated.

Commercial Drivers

Most of the phase III trials which have been conducted in this context have been funded and sponsored by pharmaceutical companies which needed to recoup investment and derive profit within timeframes limited by patents. Thus development niches requiring more extended periods of follow up, such as the non-castrate state, were commercially unattractive. Similarly, the target product profiles for these new drugs would aim to benefit larger segments of the population where possible, so combining with the standard of care presented a clear opportunity to profit from a large share of the disease population.

Thus, in the years after the introduction of docetaxel as a first line treatment for men with mCRPC, the option to combine novel drugs with the new standard of care was understandably enticing.

Combination Trials with Docetaxel

Four main classes of drug have been trialed in combination with docetaxel in phase III: the anti-angiogenics (bevacizumab, aflibercept and lenalidomide), cytotoxic chemotherapy-sensitising agents (dasatinib, custirsen, calcitriol), the endothelin antagonists (astrasentan, zibotentan) and immunotherapy vaccines (GVAX).

Anti-angiogenics

Pre-clinical studies demonstrated that Vascular Endothelial Growth Factor (VEGF) played a role in both pathogenesis and progression of prostate cancer. The expression of VEGF (Flk-1/KDR) receptors correlated with poor prognosis and was seen more commonly in the poorly differentiated tumor group [5]. Clinical studies demonstrated correlation between elevated plasma levels of circulating VEGF with

increased risk of metastatic disease progression. These data gave rise to the hypothesis that inhibition of VEGF signaling might augment current outcomes in prostate cancer [6]. In the phase II CALGB-90006 trial, 77 patients with mCRPC received bevacizumab, a humanized Immunoglobulin G monoclonal antibody to all isoforms of VEGF-A, estramustine phosphate, prednisone and docetaxel. 75 % of these men had 50 % or greater reduction in prostate specific antigen (PSA), with median progression free survival (PFS) of 8 months and overall survival (OS) of 24 months [7]. These data led into the CALGB-90401 phase III clinical trial in which 1050 patients with mCRPC were randomly assigned to receive docetaxel 75 mg/m^2 every 21 days and prednisone 5 mg orally twice per day plus either bevacizumab 15 mg/kg every 3 weeks or placebo. The primary end point was OS, secondary end points were PFS, 50 % decline in PSA, objective response (OR) and toxicity profile. The median survival for the bevacizumab arm was 22.6 months compared to 21.5 months for patients treated with placebo (hazard ratio 0.91, 95 % CI 0.78 to 1.05, log rank P = 0.181). The median PFS was superior in the bevacizumab arm (9.9 vs. 7.5 months, p = <0.001) as was the proportion of patients with OR (49.4 % vs. 35.5 % respectively, p = 0.0013). Grade 3 or 4 treatment-related toxicity was more common among patients treated with bevacizumab (75.4 % vs. 56.2 %, p = <0.001) [8]. In conclusion, this trial failed to demonstrate the case for adding bevacizumab to the standard of care.

The VENICE trial assessed the addition of aflibercept, a recombinant human fusion protein that binds A and B forms of VEGF, to docetaxel plus prednisone in men with mCRPC. This phase III trial randomised 1224 men with chemotherapy-naïve mCRPC to receive docetaxel (75 mg/m^2) three weekly plus prednisone (5 mg twice daily) and either aflibercept (6 mg/kg) or placebo intravenously every 3 weeks. The primary end-point was OS. With median follow-up of 35 months median OS was 22.1 months (95 % CI 20.3-24.1) in the aflibercept group and 21.2 months (95 % CI 19.6–23.8) in the placebo group (stratified hazard ratio 0.94, 95 %CI 0.82–1.08, p = 0.38). There was a higher rate of grade 3 and 4 gastrointestinal toxicity (30 % vs. 8 %), hemorrhagic events (5 % vs. 1.7 %), hypertension (13 % vs. 3.3 %) and treatment-related fatal events (3.4 % vs. 1.5 %) within the aflibercept compared to the placebo group [9]. Thus aflibercept was not found to increase efficacy of docetaxel in this patient group.

The phase III MAINSAIL trial investigated the addition of lenalidomide to the standard of care, docetaxel/prednisone therapy [10]. Lenalidomide is an immunomodulatory agent with anti-angiogenic properties which is routinely used in the treatment of myeloma. Pre-clinical data with lenalidomide and clinical data with the related drug thalidomide suggested a role in the treatment of prostate cancer. The MAINSAIL trial randomized 1059 men with chemotherapy-naïve mCRPC in a 1:1 ratio to receive either docetaxel (75 mg/m^2) 3-weekly with prednisone (5 mg twice daily) and either lenalidomide (25 mg) or placebo once daily for 14 days of each 21-day cycle. The primary endpoint was OS. The trial was closed early due to futility after planned interim analysis. With median follow-up of 8 months, median OS was 17.7 months (95 %CI 14.8–18.8) vs not reached in the lenalidomide and placebo arms respectively (HR 1.53, 95 % CI 1.17–2.00

p = 0.0017). The conclusion was that OS was significantly worsened by the addition of lenalidomide. The reasons for this negative effect of lenalidomide are not clear. Most of the excess deaths occurred after discontinuation of all study medication, and so it seemed unlikely that this effect was due to additional acute toxicity from lenalidomide. It has been hypothesized that the reduction in number of patients completing planned docetaxel therapy in the lenalidomide arm due to toxicity from combination therapy may be the reason for the poorer OS within this group [10].

Chemosensitising Agents

Pre-clinical trials of chemotherapy-sensitising agents highlighted the possibility of therapeutic synergy with cytotoxic agents and raised interest in clinical trials examining this principle. There have been a number of clinical trials in patients with mCRPC exploring the addition of chemotherapy-sensitising agents in combination with docetaxel/prednisone [11]. Pre-clinical data suggested possible synergy between inhibitors of the non-receptor tyrosine kinase Src and docetaxel. In addition, aberrant Src family kinase (SFK) activity has been widely implicated in prostate cancer development, cell proliferation, invasion and migration and Src may be particularly important in androgen-independent cell growth during advanced stages of disease. Src signalling is also an important pathway during normal and dysregulated bone formation and Src inhibitors may reduce morbidity from bone metastases [12]. The READY trial was a placebo-controlled phase III trial of docetaxel/prednisone with or without the Src inhibitor dasatinib in chemotherapy-naïve men with mCRPC. 1522 patients were randomly assigned to receive docetaxel (75 mg/m^2) three weekly, plus oral prednisone (5 mg twice per day), plus either dasatinib or placebo until disease progression or unacceptable toxicity. There was no improvement in OS, the primary endpoint, with medians of 21.5 months (95 %CI 20.3–22.8) and 21.2 months (95 %CI 20.0–23.4) in the dasatinib and placebo groups respectively (stratified hazard ratio 0.99, 95 %CI 0.87–1.13, p = 0.90) [13]. A randomized phase II trial of docetaxel with or without saracatinib (an alternative Src inhibitor) is underway (the SAPROCAN trial).

The SYNERGY trial explored the addition of custirsen, a second-generation anti-sense oligonucleotide (ASO) designed to bind clusterin (CLU) mRNA, resulting in inhibition of human CLU protein. Pre-clinical studies demonstrated enhanced efficacy of taxane-based chemotherapy when combined with CLU inhibition and reversal of taxane resistance [14]. A randomised phase II trial in patients with mCRPC showed that the addition of custirsen to docetaxel/prednisone prolonged OS versus docetaxel/prednisone alone (23.8 vs. 16.9 months, HR 0.50 95 % CI 0.29–0.87) [15]. These data led into the randomized, open-label phase III SYNERGY trial in which 1022 patients with chemotherapy-naïve mCRPC received docetaxel (75 mg/m^2) 3-weekly with prednisone (10 mg daily for day 1–21) with or without custirsen (640 mg for 3 loading doses followed by weekly

therapy). The trial failed to demonstrate improvement in OS (median OS of 23.4 months and 22.2 months in custirsen/no custirsen groups respectively (HR 0.93, 95 %CI 0.78–1.11, P-value = 0.42)). Further unplanned, retrospective subgroup analysis from the SYNERGY trial examined OS in 'poor' versus 'good prognosis' groups, based on 5 features previously established as prognostic factors in prostate cancer. These were Karnofsky performance status <80 %, presence of liver metastases, Haemoglobin <120 g/L, LDH >360 IU/L and PSA >150 ng/mL. Patients were deemed to have 'poor prognosis' disease if they had 3 or more of the above poor prognostic factors. OS in the 'poor prognosis' patient group was 17.0 months for those treated with docetaxel/prednisone and custirsen versus 14.0 months in docetaxel/prednisone group (HR 0.73, 95 %CI 0.59–0.90, p = 0.004). These data suggest there may be a role for custirsen in overcoming docetaxel-resistance in patients with 'poor prognosis' disease [16]. This hypothesis will be further examined in the on-going AFFINITY trial of cabazitaxel +/− custirsen in men who have had prior docetaxel for mCRPC.

ASCENT-2 compared efficacy and safety of docetaxel plus high dose calcitriol (DN-101) to docetaxel plus prednisone in an open-label phase III trial. Calcitriol had been shown to inhibit proliferation and induce apoptosis in pre-clinical tumor models, with a suggestion of synergy in combination with docetaxel therapy. A phase I trial defined the maximum tolerated dose (MTD) of 60 μg weekly based on the development of grade 2 hypercalcaemia in 2 out of 7 patients. This led into the placebo-controlled phase II ASCENT trial evaluating weekly docetaxel in combination with either DN-101 or placebo in men with mCRPC. The primary end point of the phase II trial was the number of patients with more than 50 % decline in PSA from baseline at 6 months. This trial showed a trend to greater efficacy among men receiving calcitriol (58 vs. 49 %, p = 0.16). In addition there was a significant difference in favor of DN-101 combination for tumor response (p < 0.05) and skeletal event-free survival time (p = 0.05). Further follow-up demonstrated median OS in the combination group of 24.5 months versus 16.4 months for the docetaxel-alone arm (HR 0.70, 95 %CI 0.48–10.4, P = 0.07) [17]. The prospective ASCENT-2 phase III clinical trial then enrolled 953 men with chemotherapy-naïve mCRPC who were randomly assigned to receive either docetaxel 36 mg/m^2, 45 μg DN-101 and 24 mg Dexamethasone weekly for 3 out of every 4 weeks or control (75 mg/m^2 docetaxel with 24 mg Dexamethasone every 3 weeks with 5 mg oral prednisone twice daily). The primary end point was OS. The trial was closed at planned interim analysis due to more deaths in the arm with DN-101. Median OS was 17.8 months (95 %CI 16.0–19.5) in the DN-101 arm versus 20.2 months (95 % CI 18.8–23.0) in the control arm (log rank p = 0.002). Overall survival remained inferior in the DN-101 group even after adjusting for baseline variables in patient characteristics (HR 1.33, p = 0.019). The two arms were similar in terms of serious adverse events and the most frequent grade 3 or 4 toxicities were GI (75 % of patients) and blood and lymphatic disorders (48 %). Docetaxel toxicity leading to dose modification was more frequent in the DN-101 arm (31 %) versus the control arm (15 %). The ASCENT trial group concluded that the addition of DN-101 was associated with shorter survival [18].

Endothelin Antagonists

Increased endothelin receptor A (ETrA) expression was demonstrated with advancing tumor stage and grade in prostate cancer and Endothelin-1 and ETrA interaction is critical for prostate cancer cell stimulation and osteoblastic proliferation/migration. Small molecule inhibitors of this mechanism demonstrated inhibition of metastatic development and progression in pre-clinical models and single agent trials of orally bioavailable the small molecule inhibitors atrasentan and zibotentan suggested activity, most significantly in patients with bone metastases [19, 20]. Furthermore, pre-clinical data from a bone metastasis model of prostate cancer suggested synergy between atrasentan and docetaxel. A phase II trial of atrasentan plus docetaxel/prednisone enrolled 31 patients with mCRPC and all were treated with docetaxel (60–75 mg/m^2) and atrasentan 10 mg starting on day 3. Median OS was 17.6 months (95 %CI 13.0–23.2) and median PFS 4.2 months (95 %CI 2.3–5.8) [21]. These data led into the phase III SWOG 0421 trial, which randomized (1:1) 498 patients to receive docetaxel (75 mg/m^2 three weekly) plus oral prednisone (5 mg twice daily) with either atrasentan (10 mg/day orally) or placebo for up to 12 cycles and treated until disease progression or unacceptable toxicity. Co-primary end points were PFS and OS, analysed by intention to treat. The trial was halted early in 2011 for futility after planned interim analysis. Median PFS was 9.2 months (95 %CI 8.5–9.9) in the atrasentan group and 9.1 months (95 %CI 8.4–10.2) in the placebo group (hazard ratio 1.02, 0.89–1.16, p = 0.81). Median overall survival was 17.8 months (95 %CI 16.4–19.8) in the atrasentan group versus 17.6 months (95 %CI 16.4–20.1) in the placebo group (hazard ratio 1.04, 95 %CI 0.90–1.19, p = 0.64). Three deaths in the atrasentan group and seven in the placebo group were judged to be possibly or probably due to protocol treatment. The planned interim analysis concluded that atrasentan in combination with docetaxel chemotherapy, though well-tolerated, did not improve overall or progression-free survival in men with mCRPC and bone metastases [4].

The ENTHUSE trial assessed the efficacy of zibotentan in combination with docetaxel in patients with mCRPC. This phase III trial randomized 1052 patients with chemotherapy-naïve mCRPC to receive docetaxel (75 mg/m^2 three weekly) with prednisone (10 mg daily) plus oral zibotentan or placebo once daily. The trial demonstrated no improvement in OS from the addition of zibotentan (median 20.0 vs. 19.3 months, hazard ratio 1.00, 95 %CI 0.84–1.18, p = 0.963). No significant differences were observed in secondary end points, including median time to pain progression (9.3 vs. 10.0 months, respectively) or pain response (odds ratio 0.84, 95 %CI 0.61–1.16, p = 0.283). The most common treatment-related adverse events in zibotentan-treated patients were peripheral edema (52.7 %), diarrhea (35.4 %), alopecia (33.9 %) and nausea (33.3 %). The trial group concluded that docetaxel plus zibotentan 10 mg daily did not result in significant improvement in overall survival compared to docetaxel plus placebo in patients with mCRPC [3].

Immunotherapy

There has been a great deal of recent interest in targeting the immune system to treat advanced solid tumors. Using immunotherapy to induce tumor response in prostate cancer appears to be a valid therapeutic approach from pre-clinical and phase II/III clinical trials. This has been demonstrated in the pivotal phase III trial of sipuleucel-T, an autologous cellular vaccine consisting of activated antigen-presenting cells loaded with prostatic acid phosphatase (PAP), which demonstrated median survival of 25.8 months versus 21.7 months in the placebo group. This vaccine therapy has not been fully explored in clinical trials in combination with docetaxel. Another agent investigated in combination with docetaxel is GVAX-PCa, which comprises a mixture of two irradiated allogeneic prostate cancer cell lines, LNCaP and PC-3, which constitutively express GM-CSF [22]. The phase I/II trial to evaluate the safety and immunogenicity of GVAX-PCa was performed on 55 patients with chemotherapy-naïve mCRPC. Subjects all received an intradermal priming vaccine with GVAX-PCa (5×10^8 cells) followed by 12 biweekly boosts for 6 months. Patients were allocated to receive either radiotherapy alone, GVAX-PCa with high dose boosts (3×10^8 cells) or GVAX-PCa with low dose boosts (1×10^8 cells). The median OS for the high-dose boost group was 34.9 months, 24 months for the low-dose boost group and 26.2 months for radiotherapy alone [23, 24]. These promising early data led into the phase III VITAL-2 trial, which compared GVAX immunotherapy in combination with docetaxel to docetaxel/prednisone. The study was designed to enroll 600 patients with primary end point of superiority in OS. Docetaxel (75 mg/m^2 3-weekly for 10 cycles) was given in both arms and Prednisolone (10 mg daily) given in the control arm. Patients allocated to receive vaccine were given CG1940/CG8711 (500 million cells prime/300 million cells boost doses 3-weekly for 10 cycles), followed by maintenance immunotherapy alone. The study was terminated prematurely after accrual of 408 patients due to excess deaths in the vaccine arm. There was no demonstrable difference in baseline characteristics. Fewer patients in the experimental arm completed all 10 cycles (27 vs. 37 %) of docetaxel. Overall survival was shorter in the experimental versus docetaxel/prednisone arm with median survival 12.2 versus 14.1 months [25]. Further exploratory analyses are ongoing in attempt to identify patient subgroups with preferential benefit from the investigational product.

The phase III trials exploring docetaxel combinations are summarized in Table 7.1.

Docetaxel Cytotoxic Combination Studies

Other docetaxel-based combination chemotherapy regimens with older cytotoxic agents have also been evaluated in more limited phase II clinical trials. A trial of 64 chemotherapy-naïve patients with metastatic CRPC randomised patients to either

Table 7.1 Summary of phase III combination chemotherapy clinical trials in patients with metastatic castrate refractory prostate cancer

Study	No of patients	Chemo regime	Median OS	References
TAX-327	1006	Docetaxel/prednisone	19.2 months	[2]
		Mitoxantrone	16.3 months	
Anti-angiogenic agents:				
CALGB-90401	1050	Docetaxel/prednisone/bevacizumab	22.6 months	[8]
		Docetaxel/prednisone/placebo	21.5 months	
VENICE	1224	Docetaxel/prednisone/aflibercept	22.1 months	[9]
		Docetaxel/prednisone/placebo	21.2 months	
MAINSAIL	1059	Docetaxel/prednisone/lenalidomie	17.7 months	[10]
		Docetaxel/prednisone/placebo	Not reached	
Chemotherapy-sensitising agents:				
READY	1522	Docetaxel/prednisone/dasatinib	21.5 months	[13]
		Docetaxel/prednisone/placebo	21.2 months	
SYNERGY	1022	Docetaxel/prednisone/custirsen	23.4 months	[16]
		Docetaxel/prednisone/placebo	22.2 months	
ASCENT-2	953	Docetaxel/prednisone/DN-101	17.8 months	[18]
		Docetaxel/prednisone/placebo	20.2 months	
Endothelin antagnoists:				
SWOG-0421	498	Docetaxel/prednisone/atrasentan	17.8 months	[4]
		Docetaxel/prednisone/placebo	17.6 months	
ENTHUSE	1052	Docetaxel/prednisone/zibotentan	20.0 months	[3]
		Docetaxel/prednisone/placebo	19.3 months	
Immunotherapy:				
VITAL-2	408	Docetaxel/prednisone/CG1940-CG8711	12.2 months	[25]
		Docetaxel/prednisone	14.1 months	

docetaxel (20 mg/m^2 days 1&8 of 3 weekly regimen) and vinorelbine (25 mg/m^2 on days 1&8 of 3 weekly regimen) or docetaxel (60–70 mg/m^2 three weekly) with estramustine (280 mg thrice daily days 1–5). Median survival for the docetaxel/vinorelbine arm was 16.2 months and for the docetaxel/estramustine 19.7 months. The investigators concluded that neither regimen was likely to be superior to mono-agent docetaxel, albeit that this study was limited by small numbers and lack of a direct control group [26].

The docetaxel and capecitabine doublet regimen was explored in a trial of 46 patients and demonstrated a biochmical response in 68.2 % of patients, with overall survival of 17.7 months. This did not proceed to phase III clinical trial due to perceived lack of evidence suggestive improved efficacy over docetaxel monotherapy [27]. Similarly a single-arm phase II trial of 38 patients receiving weekly docetaxel (30 mg/m^2 weekly) and epirubicin (30 mg/m^2 weekly) failed to show a

significant signal reporting that 68.4 % of patients had a greater than 50 % reduction in PSA (95 %CI 51.2–80.2 %) with median duration of response of 8.8 months (95 % CI 6.2–11.8) [28].

Prior to the results of the phase III TROPIC trial of cabazitaxel, there was no established second line therapy for patients who had progressed on the standard docetaxel/prednisone regimen. The most widely used regimes incorporated platinum compounds, which were demonstrated to have some modest activity in relatively small phase II studies conducted in patients who had progressed on docetaxel/prednisone. Kentepozidis et al published a phase II multicentre trial of 38 patients with mCRPC that had previously received docetaxel/prednisone who were then given carboplatin (AUC3 on Day 1 every 2 weeks) and paclitaxel (135 mg/m^2 Day 1 every 2 weeks). The trial demonstrated a biochemical response in 26.3 % of patients (95 %CI 12.3–40.3), stable disease in 34.2 % and progressive disease in 39.5 %. The median duration of response was 6.1 months (range 1.0–9.8) with median time to tumour progression of 3.6 months (95 %CI 2.1–5.2) and median overall survival of 9.9 months (95 %CI 6.2–3.6) [29].

A phase II clinical trial published by Ross et al. [30] investigated the role of carboplatin and docetaxel doublet therapy in the second line setting for patients who had progressed after first line docetaxel/prednisone therapy. A total of 34 patients were enrolled, all of whom had progressed within 45 days of completing docetaxel-based chemotherapy and were treated with docetaxel (60 mg/m^2, 3-weekly) and Carboplatin (AUC 4, 3-weekly) until disease progression or unacceptable toxicity. The trial demonstrated PSA reduction of greater than 50 % in 18 % of patients and measurable response in 14 % of patients. The median progression-free survival was 3 months with median overall survival of 12.4 months. These and other small phase II studies added further evidence that platinum chemotherapy may have a therapeutic role in the management metastatic CRPC in patients with progression after first line docetaxel therapy.

Lessons Learned and Future Approaches

Better Ways to Develop Combinations

As discussed, most of the failed phase III trials were conducted on the basis of positive signals gained in single arm phase II trials. History confirms that these phase II trials have not been predictive of success in phase III. The reasons for this are largely due to patient selection in phase II, and so better tools need to be used to demonstrate proof of concept for the novel combination prior to launching phase III. Increasingly randomized phase II trials designed to give preliminary comparative efficacy results are used to reduce the risk of subsequent failure in phase III [31, 32]. Furthermore, it is likely that more sophisticated pre-clinical models of prostate cancer may permit better prediction of drug efficacy in the future.

Alternative Development Opportunities

With the benefit of hindsight, it is clear that combining new drugs with docetaxel in the treatment of prostate cancer has not been the easy route to clinical impact (or profit) that was initially perceived, and all subsequent successes in the treatment of mCRPC have occurred where the development pathway has exploited other treatment niches. In particular, the post chemo niche, where placebo controlled trials with rapid survival endpoints have been possible, has proven to be a rapid route to first registration for cabazitaxel, abiraterone and enzalutamide. Furthermore, radium-223 was successfully developed in the substantial subgroup of patients who are not suitable for docetaxel. Subsequent development of both abiraterone and enzalutamide in the group of patients with minimally symptomatic disease for whom chemotherapy is not yet indicated demonstrated that placebo controlled trials could be delivered successfully in this group even where a standard of care option already existed. This latter niche was less attractive due to the extended follow-up required to demonstrate overall survival, but nonetheless has extended access to a large segment of the patient population.

A Changing Role for Docetaxel

Results from the recent CHAARTED (E3805) and STAMPEDE trials [33] have shown survival benefit if docetaxel is delivered at the time of diagnosis in men presenting with metastatic disease, with a strong suggestion that the magnitude of this benefit is very much greater than that seen in the castration resistant setting. As a result, it is likely that, in the future, many men will receive docetaxel as a first treatment. These are important findings, although the biological explanation remains unclear. However, it is possible that early treatment is important for other systemic therapies, and so it is possible that there will now be renewed interest in combining other treatments with docetaxel to maximize impact in combination with this new standard of care. Indeed, phase III trials are already underway which encompass the combination of newer anti-hormonal drugs and docetaxel in newly diagnosed metastatic disease.

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8 Cancer Vaccines in Castration Resistant Prostate Cancer—An Evolution in Design

Susan F. Slovin

Introduction

The last several years have demonstrated substantial innovations to the treatment of patients with castrate metastatic prostate cancer (CMPC) all of which have led to improved overall and/or radiographic progression-free survival. These have included an approved immunotherapy, Sipuleucel-T (Provenge™) [1], to androgen-receptor (AR) targeted agents, enzalutamide (Xtandi™) [2] and abiraterone (Zytiga™) [3], radiopharmaceutical radium-225 (Xofigo™) [4]. However, more recently, a significant change in the treatment paradigm for patients with newly diagnosed non-castrate metastatic disease to bone has led to the early introduction of docetaxel to standard hormonal regimens [5, 6]. Despite these successes, there remains a strong impetus to use or enhance the body's immune system to combat and control early micrometastatic disease that can ultimately lead to significant disease progression and death.

Recent studies in melanoma, renal cell, bladder and non-small cell lungs cancers have shown the efficacy and durability of the checkpoint inhibitors, anti-CTLA-4 (ipilimumab, Yervoy™) [7] and anti-PD-1 [8–11], -L1 (nivolumab, Optivo™; lambrolizumab, Keytruda™) [12] or the combination of ipilimumab and nivolumb [13]. These drugs not only enhance and potentiate the cellular compartment of the immune system but can be combined with other agents including standard vaccines, chemotherapy or biologic agents to lead to anti-tumor effects and disease control. However, their efficacy in prostate cancer has been suboptimal.

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Why Immunotherapy in Prostate Cancer?

Immunotherapeutic approaches for prostate cancer have been many, but the overall successes have been few. The concept of using the body's immune system to fight cancer is not new. The Toronto Globe in an article on July 17, 1925 [14] announced the plan for a vaccination against cancer based on the hypothesis that cancer was of viral etiology, yet the ideas of passive and active approaches of developing immunity toward cancer has been based on attempts to curtail infectious diseases and dates back as far as the 1800s. The rationale for immunotherapeutic approaches in prostate cancer evolved from the observations of: (1) the overexpression or under glycosylation of well-characterized cell surface molecules that are often altered self-antigens. These included the mucins and glycolipids, as well as Prostate Specific Antigen (PSA), prostatic acid phosphatase (PAP), Prostate Specific Membrane Antigen (PSMA), Six Transmembrane Epithelial Antigens of the Prostate (STEAP), Prostate Stem Cell Antigen (PSCA) among others [15, 16]. (2) Prostate cancer, unlike other cancers has a validated biomarker, PSA which can be used to monitor treatment response; circulating tumor cells (CTCs) are now being validated as a biomarker in clinical trials to determine its role in reflecting a biologic response to treatment. Interestingly, immunologic biomarkers still remain under evaluation. (3) Immunotherapy may be widely applicable to all states of the disease, from biochemical relapse through castration resistant disease, although the impact of tumor burden and the bone tropism of the disease may affect these agents. (4) These therapies can be easily integrated into standard chemotherapy regimens and may likely be potentiated when combined with chemotherapy, radiotherapy or biologic agents such as GM-CSF, IL-2 or the checkpoint inhibitors.

Lessons Learned from Immunologic Therapies

Immunotherapy as a field has used a variety of different tactics toward generating antitumor responses many of which have been suboptimal. These approaches have varied the antigen(s) used, in addition to using unique adjuvants to immune response as well as novel antigen-antibody drug conjugates. These antigens have been diverse and have included altered self-antigens including the mucins (MUC-1, MUC-2), glycolipids (Globo H), gangliosides (GM-2), as well as protein and peptides of PSA, PSMA and PAP [15, 16]. The strategies used included xenogeneic DNA, viral replicon particle PSMA [17] and PSA [18] vaccines, in addition to immunologic adjuvants such as QS-21 [15, 18] or alhydrogel [18], or the incorporation of co-stimulatory molecules into the construct. Despite these novel antigens and approaches, increasing doses of vaccine did not correlate with augmentation of immunogenicity and lower doses were sometimes more immunogenic. There was no potentiation of T cell responses. In addition, an immunologic signal or even anti-tumor effect radiographically was not immediate and could take up to six-months to show some effect.

What has been gleaned from all these approaches is that these vaccines could induce high titer antibodies specific for the immunogen, ie, antigen used within the vaccine construct, as well as modulated the post versus pre-treatment PSA slopes. However, neither antibody induction nor changes in PSA slopes could be associated with any changes in the biology of the cancer. No clear cut immunologic endpoints have been identified for use in clinical trials which have made trial design difficult; there have been no standardized or validated immunologic biomarkers that were easily measurable and could reflect biologic change in the disease. These biomarkers may need to be specific for the particular immune therapy used or may be specific to the disease and the means by which the disease is monitored [19].

Prostate cancer remains a unique solid tumor malignancy in that it has not been considered an "immunologic cancer" like melanoma or renal cell cancers where spontaneous remissions can occur, or disease can remit at metastatic sites following resection of the primary lesion. Melanoma has for years been the example of an immunologically driven cancer, responsive to autologous and ganglioside vaccines and more recently, serving as an ideal model of disease response for the checkpoint inhibitors.

More recently, new data have been presented that the hypermutated status of melanoma [20] (Fig. 8.1), renal and non small cell lung cancers can make them more susceptible to these novel agents, ie, the checkpoint inhibitors, and that certain mutations, even in other diseases, may be amenable to checkpoint inhibition. This was recently reported in non-melanoma patients whose cancers had MSH-1

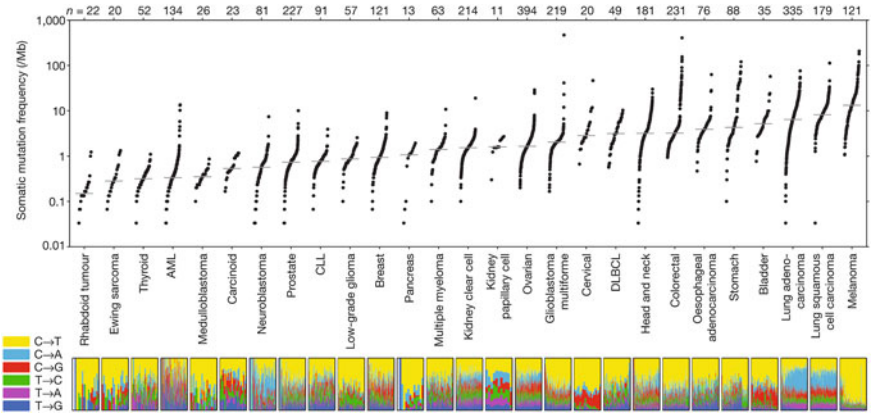

Fig. 8.1 The mutational frequencies of solid tumors vary considerably with prostate being much less than others, ie, melanoma, lung cancer, and bladder cancers. Seen here is the ordered somatic mutation frequencies observed in exomes from 3083 tumor-normal pairs. A *dot* corresponds to a tumor-normal pair, with vertical position indicating the total frequency of somatic mutations in the exome. The lowest frequencies (*left*) are found in hematological and pediatric tumors; the highest (*right*) in tumors induced by carcinogens such as tobacco smoke and UV light. Mutation frequencies vary more than 1000-fold between lowest and highest mutation rates across cancer and also within several tumor types. Reprinted from Lawrence et al. [22]. With permission Nature Publishing Group

mutations and were found to be highly responsive to checkpoint inhibition [21]. As seen in Fig. 8.1 [22], prostate cancer does not have the same level of mutations as these other cancers suggesting that this may be one mechanism by which prostate cancers may be suboptimally responsive to checkpoint inhibitors.

The Development of Prostate Cancer Immunotherapy

A review of several large phase III clinical trials in prostate cancer patients with castrate metastatic disease has demonstrated numerous failures and limited successes. Among the former was the VITAL-1 and VITAL-2 clinical trials. The trials were based on strong preclinical and clinical data of an immune approach using prostate cancer cell lines that were genetically modified to secrete the immune stimulatory cytokine, granulocyte-macrophage colony-stimulating factor (GM-CSF), and then irradiated to prevent tumor cell proliferation. The product was prepared as both an autologous as well as an allogeneic vaccine [23–25]. VITAL-1 was a phase 3 clinical trial of GVAX immunotherapy in patients with asymptomatic metastatic hormone-refractory prostate cancer. The trial was fully enrolled in 2007 with 626 patients and compared GVAX immunotherapy to docetaxel plus prednisone. The Sponsor terminated the trial based on the results of a previously unplanned futility analysis conducted by the study's Independent Data Monitoring Committee (IDMC). This indicated that the trial had less than a 30 % chance of meeting its predefined primary endpoint of an improvement in overall survival.

The second of two phase 3 clinical trials of GVAX immunotherapy for prostate cancer (VITAL-2), compared GVAX immunotherapy in combination with docetaxel with docetaxel plus prednisone in patients with advanced-stage prostate cancer [26]. The Sponsor terminated the trial as recommended by its Independent Data Monitoring Committee (IDMC) which, during an interim safety review noted an imbalance in deaths between the two treatment arms. VITAL-2 enrolled 408 patients. The IDMC based its recommendation on the 76 of 114 deaths which occurred in the GVAX plus docetaxel combination arm compared with 47 deaths occurred in the chemotherapy only control arm. While an assessment of potential imbalances between the two arms of the study such as baseline characteristics and prognostic factors, as well as other treatment variables were reviewed, no one factor accounted for these observations. However, a major point of contention centered on the overall trial design which used docetaxel weeks on a three week on, one week off schedule that was not in keeping with the standard of care dosing of the drug which was every three weeks. The concerns with this trial led to a previously unspecified futility analysis of VITAL-1. Despite the lack of success in prostate cancer, its use in pancreatic cancer may be better tolerated approach than s conventional chemotherapeutic agents. Here, it involves two different anticancer vaccines: GVAX Pancreas followed by CRS-207 [27]. The GVAX approach follows the same strategy as that used in the earlier prostate cancer trials and uses pancreatic cancer cells that have been genetically modified to secrete GM-CSF. In this case,

GVAX is given with low-dose cyclophosphamide to inhibit regulatory T cells and enhance efficacy. A second vaccine, CRS-207, is live-attenuated *Listeria monocytogenes* (Lm) which has been genetically modified to not replicate but still provide additional immunogenicity by stimulating an immune response against mesothelin, a molecule which serves as a tumor-associated antigen on pancreatic tumor cells [27].

The disappointing results of VITAL-1 and -2, led investigators to consider alternative options that could involve some strategies whereby the body's own cells could be used either directly or indirectly to fight cancer. Immunologists have known for years that dendritic cells (DCs) otherwise known as antigen processing cells (APCs) subserved a significant function by acting as scavengers to pick up, digest and process a variety of biologic remnants that can the be processed and presented to T cells via the T cell receptor (TCR) in the context of the major histocompatibility complex (MHC) in preparation for engagement with the T cells. These materials could be discarded cell membranes, necrotic cells post radiation or chemotherapy, all of which can be seen as "foreign" to the immune system. In many cases, they may be altered "self antigens" and not immunogenic.

The engagement of DCs, the presentation of digested antigens into nonomers, ie 9-amino acid peptides in the context of MHC, is insufficient alone for engagement of the APC and T cell; co-stimulatory molecules are needed to facilitate the completion of the interaction and cell-to-cell signal. Assembly of major histocompatibility complex (MHC) molecules, which present antigen in the form of short peptides to T lymphocytes, occurs in the endoplasmic reticulum(ER). Once assembled, these molecules can travel from the ER to their final destination. The recognition of the MHC:peptide complex by the TCR constitutes the first signal delivered to the T cell. CD4/CD8 are the co-receptors for MHC molecules and also engaged in this collaboration. A second signal is then delivered by the engagement of co-stimulatory molecules on the APCs, ie, CD80 (B7-1)/CD86 (B7-2), CD54 (intracellular adhesion molecule-1, ICAM-1). These molecules bind to their ligands on the T cells, ie, to CD28 (B7 protein) and CD11a ($\alpha 2\beta 2$ integrin)/CD18(LFA-1), respectively, and along with the T cell engagement, leads to T cell activation. It is important to remember that only "professional" APCs, ie, DCs, macrophages and B cells express these co-stimulatory molecules [28]. The exception to the rule is that "non-professional cells" such as fibroblasts and endothelial calls lack expression of these molecules and do not play a role in the direct engagement and activation of T cells with APCs. It should be noted that there is significant cross-talk between the APCs and the T cells as the engagement process causes an upregulation of the expression of the co-stimulatory molecules and likely may induce expression of other molecules or cytokines. Cytotoxic-lymphocyte antigen-4 (CTLA-4) is also expressed on T cells and serves as a ligand for CD80/86. It acts as a "brake" to prevent non-specific T cell activation, and makes the T cell abort the interaction between MHC:peptide and TCR signals [29, 30].

The concept that DCs can be effective agents on their own led to the first FDA approval of an immunotherapy not only for prostate cancer but the first in the setting of a solid tumor malignancy. This cellular product therapy, Sipuleucel-T (Provenge™)

[1] was approved based on several phase I and II clinical trials which initially did not meet their original endpoints of time to progression but was found to meet the secondary endpoints of overall survival. In the phase III 352 patient clinical trial randomized 2:1 in favor of cellular product versus placebo, the sipuleucel-T group showed a relative reduction of 22 % in the risk of death as compared with the placebo group (hazard ratio, 0.78; 95 % confidence interval [CI], 0.61–0.98; $P = 0.03$). This reduction represented a 4.1-month improvement in median survival (25.8 months in the sipuleucel-T group vs. 21.7 months in the placebo group). The survival benefit led to its approval for patients with asymptomatic or minimally symptomatic castrate metastatic prostate cancer. Patients were leukapheresed and peripheral blood mononuclear cells sent to a central facility where they were co-cultured with a fusion protein of prostatic acid phosphatase (PAP) and Granulocyte-Macrophage Colony Stimulating Factor (GM-CSF). After a 48 h incubation, cells were reinfused back to the patient every two weeks for a total of 3 infusions.

The end product was comprised largely of CD54+ cells that were activated by the fusion protein [31]. While well-tolerated overall, infusion reactions manifested as fever, headache, flu-like illness, myalgia, hypotension, or shortness of breath occurred rarely but were managed easily with steroids and acetaminophen. Despite a crossover design, the majority of patients in both study groups received docetaxel after study treatment. Based on a sensitivity analyses, there did not appear to be sufficient evidence that between-group differences in the use of docetaxel could account for the observed treatment difference relative to overall survival. The limitations of the study included the crossover design carried over from the original studies and the fact that patients were monitored for about 6 months. Given current observations with other therapies, the suggestion was that there could be a delayed antitumor effect in the setting of continued rises in PSA and that therapy should be delayed if possible. However, patients were often uncomfortable awaiting any impact on PSA or radiographic disease and would want to proceed with other therapeutic interventions.

The early success and enthusiasm for Sipuleucel-T, its safety profile and relative ease of administration have subsequently been dampened by its limited efficacy, the unknown time to its antitumor effect, if any, and its competition with the newer AR-directed therapies such as enzalutamide and abiraterone. However, the efforts to evaluate potential vaccines continue to be based the fact the vaccines worked by promoting type 1 or type 2 immune reactions. In type 1 immune reaction, T helper type 1 (Th1) lymphocytes secrete interleukin-2 (IL-2), interferon gamma, and lymphotoxin-alpha and could facilitate phagocytic activity. Type 2 immunity involves Th2 cells that secrete IL-4, IL-5, IL-9, IL-10, and IL-13 and is engendered by high antibody titers. More recent analyses of the large IMPACT and PROACT trials have led to a more thorough evaluation of the immune samples in an effort to determine the mechanism of action of Sipuleucel-T™ [31–33].

Two recent studies in human cancer have validated the research into peptide-based vaccines being able to become immunogenic and may do so by epitope spreading [34]. One example, involved a vaccine using ERBB2 (HER-2/neu), a self-antigen that is overexpressed in 15–30 % of human adenocarcinomas [35]. Patients with

ERBB2-overexpressing breast or ovarian tumors received ERBB2 peptides given with GMCSF which had enhanced immunogenicity based on observations in a preclinical model. This approach in rats elicited $CD4^+$ T-cell responses to the intact protein. There was no evidence of ERBB2-specific responses prior to vaccination. Surprisingly, following immunization with the specific peptides, patients acquired immune responses not only to ERBB2 peptide but also to other peptides not originally included within the vaccine construct, hence the mechanism of "epitope spreading" (Fig. 8.2). In another study of breast/ovarian cancer, patients were injected

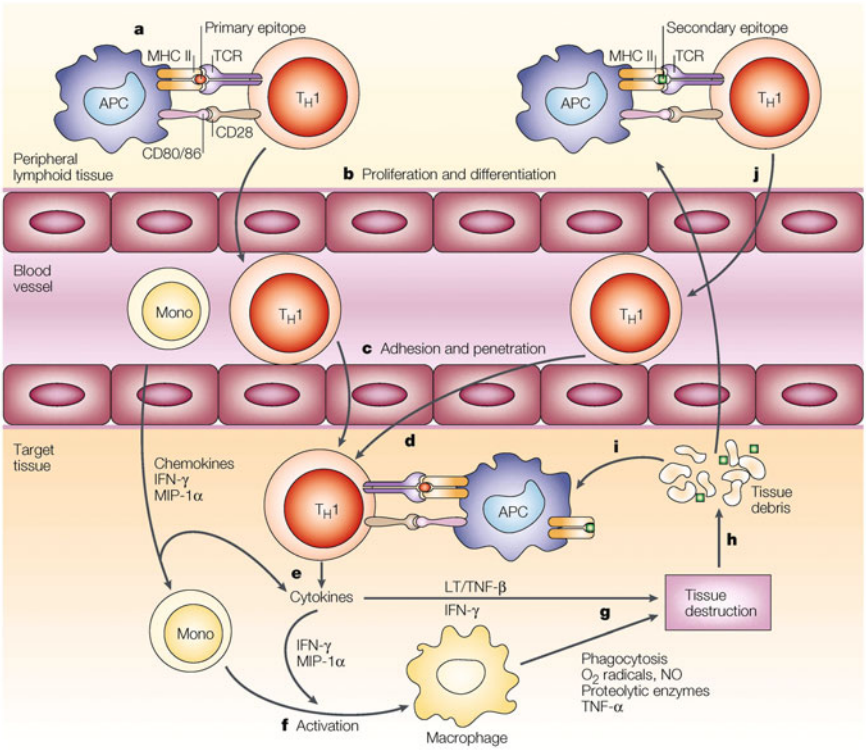

Fig. 8.2 Cartoon showing the concept of antigen spreading. Presentation of the primary epitope (the immunodominant self or viral epitope) occurs in peripheral lymphoid tissue (**a**), resulting in activation and differentiation of autoreactive T_H1 cells (**b**). The activated T_H1 cells migrate (**c**) into the target tissue, where they encounter antigen presented by resident APCs (**d**). After antigen re-stimulation, the pathologic T_H1 cells release a cascade of chemokines and cytokines (**e**), leading to recruitment of additional mononuclear phagocytes from the peripheral blood, which are activated along with resident APCs (**f**). Activated mononuclear cells then lead to bystander tissue destruction (**g**) via phagocytic mechanisms and release of TNF-α, proteolytic enzymes, NO and O_2 radicals. The tissue debris (**h**) is processed and presented on resident and peripheral APCs (**i**), leading to the activation and differentiation of a second wave of T_H1 cells (**j**), which can re-enter the tissue and cause additional tissue destruction. *APC*, antigen presenting cell. Reprinted from Vanderlugt and Miller [34]. With permission from Nature Publishing Group

with autologous dendritic cells pulsed with ERBB2- or mucin-1-derived peptides. Data from several patients in this study showed that vaccination with a single tumor antigen could induce cytotoxic T cell reactivity towards several tumor antigens by virtue of epitope spreading in vivo [35–37]. These results suggest that epitope spreading may increase the efficiency of protein vaccination.

Further extrapolation of these preclinical and clinical studies can be extrapolated into other approaches [38]. It has been proposed that antigen spreading (Fig. 8.2) may be the presumed mechanism of action of this Sipuleucel-T. As part of the analysis, IgG titers were elevated against multiple secondary antigens, including PSA, KLK2/hK2, K-Ras, E-Ras, LGALS8/PCTA-1/galectin-8, and LGALS3/galectin-3, following treatment with sipuleucel-T ($p < 0.01$), but not in controls [39]. IgG responses (defined as ≥2-fold elevation post-treatment) occurred in ≥25 % of patients were induced by 2 weeks after sipuleucel-T treatment, and persisted for up to 6 months. Interestingly, IgG responses to PSA and LGALS3 were associated with improved OS in sipuleucel-T-treated patients from IMPACT ($p \leq 0.05$). The authors concluded that Sipuleucel-T induced humoral antigen spread mCRPC patients and that IgG responses were associated with improved OS in IMPACT.

Along the same developmental path as Sipuleucel-T™, PROSTVAC® has also demonstrated a survival benefit based on a phase II trial [40]. This was a multi-center phase II trial of 125 patients with CMPC and were randomly assigned. PROSTVAC-VF® comprised two recombinant viral vectors, each encoding transgenes for PSA, and three immune costimulatory molecules (B7.1, ICAM-1, and LFA-3). The vaccinia-based vector was used for priming followed by six planned fowlpox-based vector boosts. Patients were allocated (2:1) to PROSTVAC-VF® plus GM-CSF or to control empty vectors plus saline injections. The approach was safe; 82 patients received PROSTVAC-VF® and 40 received control vectors. The primary end point was progression-free survival (PFS); this was similar in both groups ($P = 0.6$). Those patients in the PROSTVAC-VF® arm after a 3 year follow up had better OS, with 25 (30 %) of 82 patients still alive compared with 7 (17 %) of 40 controls, longer median survival by 8.5 months (25.1 vs. 16.6 months for controls), an estimated hazard ratio of 0.56 (95 % CI, 0.37–0.85), and stratified log-rank $P = 0.0061$. A phase III multi-national registration trial of PROSTVAC-VF®, using a heterologous prime-boost strategy with vaccinia and fowlpox viral vectors encoding PSA, has since closed after reaching its targeted accrual and is under analysis. More recently, a phase II trial [41] evaluated the safety, clinical efficacy and immunologic data in 10 of 144 patients with CRPC who had a predicted survival of at least 18 months. Of these, 8 of 10 patients were randomized to receive either docetaxel alone (Arm B, n = 2) or treatment with PROSTVAC-VF® (days 1, 15, 29, 43, 57) to be followed by docetaxel (Arm A, n = 6) beginning at month 3. The primary endpoint of the trial was overall survival, and secondary endpoints included time to radiographic progression and immunological response. The limitation in the number of patients reflected early closure of this trial due to slow accrual after a 13 month interim evaluation. Of the 6

patients treated on the chemotherapy alone arm, 2 who received vaccine followed by docetaxel demonstrated a >50 % PSA decline, one of whom had this occur during the actual treatment with the vaccine prior to chemotherapy. Significant PSA-specific CD4+ and CD8+ T-cell responses and IgG antibody responses specific for PSA were not detected. Unfortunately, overall survival could not be assessed. In the quest for biomarkers of immune responsiveness, there have been multiple parameters studied in a variety of trials in an attempt to ascertain a biologic response by the immune therapy even if lacked radiographic confirmation.

An interesting study by Campbell et al. [42], found that the PROSTVAC-VF® vaccine could induce humoral responses but to a carbohydrate on the poxvirus, the Forssman disaccharide (GalNAcα1–3GalNAcβ) rather than inducing T cell responses as it was originally designed to do. These responses had a statistically significant correlation with overall survival in two independent sample sets (P = 0.015 and 0.008), respectively, in a cohort of greater than 100 patients. The anti-Forssman humoral responses correlated with clinical outcome in another study of PROSTVAC-VF® [43]. The survival correlation was specific to the vaccine cohort alone. These results suggest that an anti-glycan antibody response could be viewed as an early biomarker of a favorable response to PROSTVAC-VF®. As such further inquiry into this observation is needed.

Other Novel Constructs to Induce Immunologic Signals

Another unique approach is a prostate-cancer vaccine containing self-adjuvantated mRNA (RNActive®) [44] encoding the known prostate cancer antigens PSA, PSCA, PSMA, and STEAP. The construct of an m-RNA-based vaccine involves the incorporation of both free and protamine-complexed mRNA. The optimal expression of the encoded antigen is maintained via innate immune stimulation with a built-in adjuvant that is at partly mediated via Toll-like receptor 7 activation [45, 46]. CV9103 encodes full-length antigens and appears to be able to induce an immune response against all epitopes contained within the target protein in the absence of HLA restrictions. The lack of HLA and the inclusion of multiple antigens, likely can reduce the risk of tumor immune escape due to loss of expression of individual antigens, to increase the clinical efficacy by inducing a broader immune response and to provide immune responses against antigens present in the individual tumor in a higher number of patients. This phase I/IIa study evaluated the safety and immunogenicity of 5 intradermal injections of CV9103 in patients with advanced CMPC. Three dose levels of total mRNA were tested in Phase I in cohorts of 3–6 patients to determine a recommended dose, with 32 patients in the phase II, being treated at the recommended dose of 1280 μg. The primary endpoint was safety and tolerability; secondary endpoints included induction of antigen specific immune responses monitored at baseline and at weeks 5, 9 and 17. Of 33 patients, 26 were treated at 1280 μg; 15 of 33 patients developed an immune response directed against multiple antigens. One patient showed a confirmed PSA response.

In the subgroup of 36 metastatic patients, the Kaplan-Meier estimate of median overall survival was 31.4 months [95 % CI: 21.2; n.a]. The investigators plan to study the addition of other known antigens such as PAP and MUC-1.

Antibodies in Prostate Cancer: Antibody-Drug Conjugates (ADCs) and the Checkpoint Inhibitors

Monoclonal antibodies have been used to target cancer through a variety of different approaches including as single agents and together as a conjugate with a prodrug or with chemotherapy or radiation therapy. As pointed out by Teicher and Chari [47], there is substantial concern for designing a maximal conjugate that can enhance drug delivery within the cell, drug clearance both in the cell and the peripheral circulation, inherent toxicity to other cell populations, and, in the case of radioactive conjugates, assuring that the amount of radiotoxicity is not marrow toxic for some bone trophic diseases, for example prostate cancer. For most conjugates, it is important that the linkage have an average of 3–4 drug molecules per antibody molecule. This seems to be optimal because it diminishes the percentage of unconjugated antibody, maintains the circulating half-life near that of the naked antibody, maintains antibody binding to the target protein, and delivers sufficient numbers of cytotoxic molecules to the target cell to be lethal [47].

The greatest impediment though may be that antigen expression still needs to be optimized in order for targeting to occur. Many antigens such as PSMA are upregulated which prostate cancer becomes androgen independent [48]; antigen shedding as the tumor progresses may lead to altered or diminished expression thereby making drug targeting less successful than originally anticipated by the initial immunohistochemistry or tissue microarrays.

Maximizing Antibody/Drug Conjugates

The construction of antibody conjugates has shown itself to be an effective method to increase the therapeutic index of highly potent cytotoxic agents. Given the potential for added toxicity based on the potency of the drug component with the construct, it has become necessary to learn how to schedule these as one would for standard cytotoxic therapies; dosing once every 3 weeks has become commonplace [49–51] as patient compliance often declines with more frequent dosing. For most preparations of dual conjugates, the linker must remain stable in the bloodstream, (plasma compartment) to avoid premature release of the drug. The linker should not induce side effects within the patient as some linkers can cause peripheral neuropathy or visual changes by virtual of their clearance. Similarly, once within the cell, the drug must either be activated or already active to initiate a cascade of cellular events which will presumably lead to cell killing. Antigen expression must be abundant and on the external aspect of the cell. Characteristics of the ADC target

such as copy number, heterogeneity and specificity of expression, internalization rate, and intracellular trafficking can be used to guide the selection of linker (cleavable or non-cleavable) and the potency and characteristics of the drug released (cell permeable or impermeable) [49, 50].

The small-molecule drugs that have been widely used for antibody-drug conjugates have mostly targeted tubulin or DNA. Also included are thee maytansinoids [52–54] and dolastatin analogs target tubulin, and both suppress microtubule dynamics [52–57]. Examples include ASG-5ME, an antibody-drug conjugate that delivered a small molecule microtubule disrupting agent MMAE. It was thought that that ASG-5ME was internalized and trafficked through the endocytic pathway prior to cell surface binding. Within the lysosomes, there was proteolytic cleavage of the vc linker and release of unmodified MMAE that becomes available for tubulin binding. It mediated potent dose dependent cell cytotoxicity in vitro.

Other antigens incorporated into drug conjugate constructs include PSCA [58, 59], STEAP [60] and PSMA [61–68] PSMA has served as a focal point for various immunologic strategies including naked PSMA DNA vaccines [62], and PSMA protein conjugate vaccines [59–61], PSMA-VRP vaccines [63], as well radiolabeling of J591 [66–68] monoclonal antibody against the external domain of PSMA using several radionuclides including ^{90}yttrium, ^{177}lutetium, ^{89}zirconium, respectively [66–68]. These conjugates have all shown exquisite targeting to sites of active disease with impact on pain control in some patients. Clinical trials continue with these agents.

The checkpoint inhibitors represent a class of drugs that are negative regulators of T cell proliferation. Although sipuleucel-T still remains an active choice for patients with asymptomatic or minimally symptomatic CRPC, the stunning and durable responses seen by the checkpoint inhibitors, ipilimumab [7, 69, 70] and nivolumab [8–11], in several malignancies. While studied in prostate cancer [71, 72], responses were suboptimal compared with those seen in melanoma, non–small cell lung, renal, and bladder cancers. Why prostate cancer behaves differently is unclear. The phase I/II report [71] of a dose-escalating study of ipilimumab with and without radiation therapy to a single site in bone showed stable disease and several dramatic and durable responses. This provided the impetus for the recently reported phase III trial [72] for patients who progressed after docetaxel treatment and were randomly assigned 1:1 to receive bone-directed radiotherapy (8 Gy in one fraction) followed by either ipilimumab 10 mg/kg or placebo every 3 weeks for up to four doses. Non-progressing patients could continue to receive ipilimumab at 10 mg/kg or placebo as maintenance therapy every 3 months until disease progression, unacceptable toxic effect, or death. This came close to, but did not meet, its endpoint of OS. An exploratory and post-hoc subgroup analysis, noted an OS benefit for ipilimumab in a subset of patients without visceral metastases, with normal or mildly elevated alkaline phosphatase, and without anemia. This suggested that ipilimumab may still be effective but in patients with more favorable prognostic features. Despite this, there is still consideration to trying to maximize their effectiveness in prostate cancer via other combinatorial approaches.

"Targeting" the Future

Genetically engineering T cells may offer an alternative to more conventional antibody based strategies by remedying the biologic limitations that constrain the antitumoral functions of normal T cells [73–77]. Unlike the physiologic T-cell antigen receptor (TCR), chimeric antigen receptors (CARs) encompass immunoglobulin variable regions or receptor ligands as antigen-recognition elements, thereby permitting T cells to recognize cell surface tumor antigens in the absence of HLA expression (Fig. 8.3a, b). T-cell activation is mediated by the cytoplasmic domain of the CAR, which is typically derived from the CD3-zeta chain or the FcRI-gamma chain (Fig. 8.3a, b) [73, 0]. Sadelain's group [70, 73] has demonstrated that the zeta chain–based CARs could induce strong activation capable of sustaining T-cell proliferation and permitting secondary antigenic re-stimulation in vitro provided that antigen was presented in the context of CD28-mediated costimulation [73, 77]. In an effort to determine if T cells, particularly human T cells,

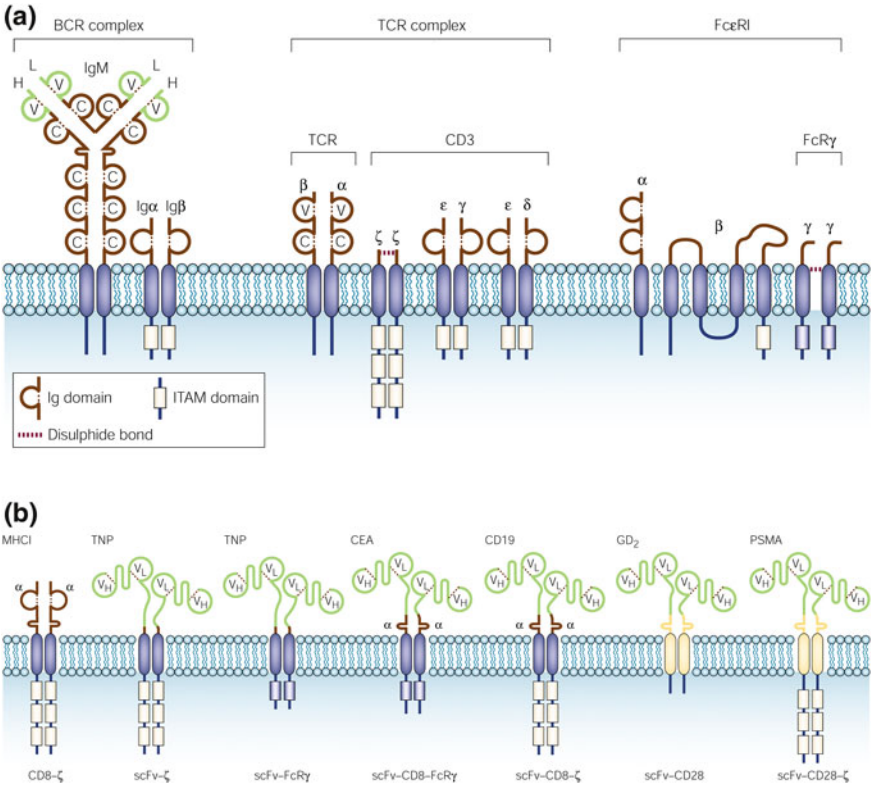

Fig. 8.3 **a, b** Panel A illustrates the anatomy of the T cell receptor and its various chains serving as the framework for CAR constructs. Panel B represents the constructs designed with specificity for a particular antigen, ie, PSMA, and others. Reprinted from Sadelain et al. [73]. With permission from Nature Publishing Group

expanded in this manner could mediate tumor eradication in vivo and if further in vivo costimulation would be needed to sustain their function, three tumors models using severe combined immunodeficiency-beige/beige mice were developed that showed that PSMA-targeted T cells could effectively eliminate prostate cancer. T cells were transduced with Pz1, a CAR-targeting human PSMA [78–80]. The Pz1 receptor encompasses the zeta chain of the CD3 complex as its activation domain and specifically redirects in vitro cytolysis again PSMA-positive tumor cells lines. The tumor models included orthotopic, subcutaneous, and pulmonary diseases; tumor eradication was directly proportional to the in vivo effector-to-tumor cell ratio. Serial imaging studies revealed that the T cells had to survive for at least 1 week to induce durable remissions [78]. The administration of Pz1-transduced T cells induced objective responses in all mice and cured a substantial fraction of them. Based on the favorable responses, several clinical trials have been actively pursuing this approach using unique combinations with constructs that encompass unique vectors or are given in combination with cytokines. Although these approaches have been well-tolerated—stable disease has been seen but in a majority of cases—a cytokine release syndrome is observed following administration of the cells suggesting T cell activation [81, 82]. Achievement of maximal responses in solid tumor may depend on the nature of the vector, the ability of cells to migrate to and persist at the tumor site, incorporation of a multi-antigen construct with molecules such as PSA, PAP, PSMA, or prostate stem cell antigen or delivering a sufficient number of cells to reach the tumor site without causing worsening toxicities [80–82].

Conclusions

This is an exciting time to for immunotherapy to become part of the prostate treatment algorithm; some consideration must be made in devising strategies whereby these therapy can be rationally integrated into the current and future treatment arenas in prostate cancer. Combinations of immunotherapy with chemotherapy, radiation, radiopharmaceuticals or the newer AR-directed therapies offer opportunities for development. How to best determine whether or not an immunotherapy impact on the biology of the cancer in the absence of radiographic change may heavily rely on the standardization of immunologic biomarkers that can be associated with some form of treatment benefit. While melanoma leads the way in this area, nevertheless there is a need to fulfill this exploration in a wide range of diseases. Immunogenomics will likely play an integral role and may offer a new outlook on how to good forward.

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Abiraterone for the Treatment of mCRPC

9

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Introduction

Huggins and Hodges first identified that prostate cancer (PCa) was a hormone-driven disease and established as standard treatment androgen deprivation by surgical castration or medical castration (administration of estrogens) [1]. Virtually all metastatic PCa respond to castration but disease invariably progresses after a median of 18–24 months. This state was initially characterized as "hormone insensitive" or "hormone resistant" PCa but these terms have now been abandoned for the term "castration-resistant PCa (CRPC)".

It was hypothesized early that the effect of castration could be augmented by suppression of adrenal androgen biosynthesis as after castration androgens of extragonadal origin were still existent at about 10 % of the pre-castration level. For that purpose adrenalectomy [2] and hypophysectomy [3] were attempted with poor

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outcome but provided evidence of activity and support for the rationale of suppression of extra-testicular androgens. Later this was pursued with administration of corticosteroids to suppress the hypothalamic ACTH axis [4]. Ketoconazole, an imidazole antifungal, was subsequently used [5] which inhibits enzymes involved in adrenal steroid synthesis, including CYP17, albeit non-selectively and incompletely [6], with PSA responses in approximately 60 % of patients [7], but also grade 3–4 toxicities in approximately 20 % of them [8].

Early Development of Abiraterone

Ketoconazole's efficacy in CRPC sparkled the development of more potent and selective inhibitors of CYP17A1 [9, 10]. Pioneering work at the ICR in Sutton, UK, identified in the early 1990s that both steroidal [11] and nonsteroidal pyridyl esters [12, 13] exhibited selectivity for CYP17A1 inhibition.

The steroidal inhibitors are derived from the natural substrates of CYP17A1, pregnenolone or progesterone [14], bind irreversibly to CYP17A1 and have increased inhibitory properties over many nonsteroidal agents. The 3-pyridyl analogues (Figs. 9.1 and 9.2) result in more potent inhibition of CYP17 relative to the 2-pyridyl and 4-pyridyl analogues [12] and the 16,17-double bond adjacent to the pyridyl residue is necessary for the irreversible binding [15].

One such steroidal compound, CB7630, later known as abiraterone acetate (AA) or JNJ-212082 is a 3ß-O-acetated version and a prodrug of CB7598, with better bioavailability and easier formulation than the 3ß-hydroxy compound (Figs. 9.1 and 9.2) and its administration in mice was able to decrease circulating levels of testosterone [10].

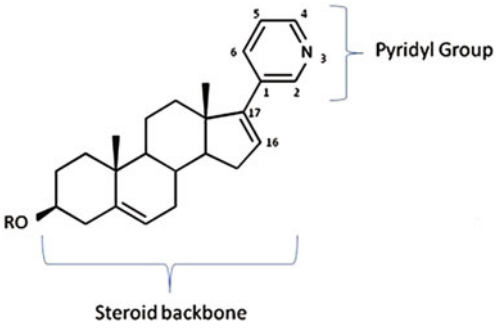

Fig. 9.1 Abiraterone structure. Abiraterone (CB7598) for R=H and abiraterone acetate (CB7630) for R=Ac. Adapted from O'Donnell [16]. With permission from Nature Publishing Group

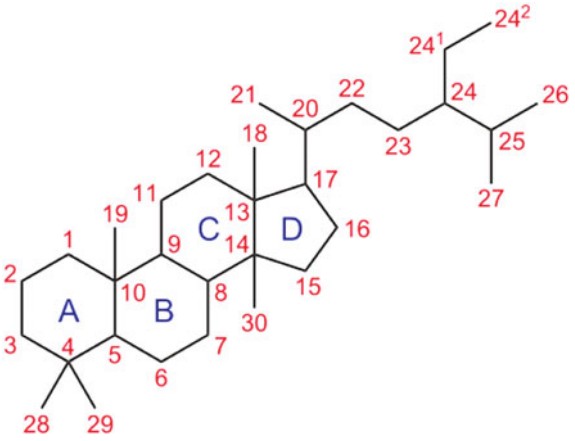

Fig. 9.2 The parent ABCD steroid ring system (hydrocarbon framework) is shown with IUPAC-approved ring lettering and atom numbering

Phase I and II Development

In 2004 the first in human trial of AA in PCa was reported and consisted of three small phase I studies of AA without the use of concomitant corticosteroids in castrate and non-castrate patients [16]. Administration was continuous in one of the arms and once-off in the other two and AA was dose-escalated from 10 to 800 mg with pharmacokinetic (PK) studies indicating good bioavailability and supporting once-daily dosing. AA was well tolerated without documented grade 3–4 toxicities. Abiraterone plasma concentrations were detected at doses of AA ≥ 200 mg with consistent effects on testosterone levels when dose-escalated to the 500 and 800 mg dose level. In castrate males suppression of testosterone was sustained, while in non-castrate males a compensatory increase in LH levels overcame inhibition of gonadal testosterone synthesis. Boehringer Ingelheim, to which abiraterone was licensed at that time, suspended further clinical development despite these initial data. Later on, the interest in abiraterone was re-sparkled, probably because of accumulating data supporting an important role of CYP171A in castration resistance.

In 2008 and 2010 two additional phase I trials were reported respectively [17, 18]. In the first study, twenty-one chemotherapy-naive CRPC patients were dose-escalated from 250 to 2000 mg once daily, with 3 patients per cohort and 6 additional patients in the 1000 mg group to complete PK and pharmacodynamic (PD) studies. The recommended dose for the phase II development was determined at 1000 mg OD as above that level there was a plateau of the endocrine effect of AA. In the 2010 study 33 chemotherapy-naïve CRPC patients were enrolled, of which 19 had received prior ketoconazole therapy. Patients were dose-escalated from 250 to 1000 mg once daily; all patients received a single dose at day 1, and continuous dosing from day 7 with fed and fasted cohorts at each dose level. PSA

and objective responses were commonly witnessed and warranted further evaluation.

The first phase II study on AA was reported in 2009 on 42 chemotherapy-naïve patients [19], and was a phase II expansion of the UK phase I study reported in 2008 [17]. Encouraging PSA declines of ≥50 % were seen in 28 of 42 CRPC patients, with objective responses by RECIST in 9 of 24 patients with measurable disease. In this study the median time to PSA progression was 225 days (95 % CI, 162 to 287 days). Subsequently further phase II trials were performed which are summarized in Table 9.1, both in the pre- as well as in the post-chemotherapy setting and strengthened proof of activity across the whole spectrum of mCRPC with a frequency of PSA declines of ≥50 % between 36 % and 51 % which appeared higher in ketoconazole-naïve rather than in ketoconazole-pretreated patients. In the first reported study AA was given without the use of concomitant steroids [20] whereas later prednisone 5 mg was prescribed twice daily from the beginning of the treatment [21].

Pharmacokinetics and Pharmacodynamics of Abiraterone and Interaction with Other Drugs

Pharmacokinetics

PK data of Abiraterone are available from the first phase I studies [17, 18] as well as from two post-licensing studies: a PK study in healthy men [22] and a population PK analysis with 359 subjects including 62 healthy volunteers [23].

Pharmacokinetics of Abiraterone are strongly influenced by food and therefore it is administered routinely in the fasted state, 2 h after a previous meal and 1 h before the next meal. Following oral administration AA is converted to its active metabolite Abiraterone by rapid hydrolysis taking place in the intraluminal environment of the intestine [24] but also in the liver involving hydroxylation mediated by esterases [25]. Therefore, AA is below detectable levels in the plasma [22] and Abiraterone is detectable instead at doses of AA ≥200 mg. Abiraterone reaches its maximum concentration (C_{max}) of 1.2–5 μM in approximately 1–2 h in fasting patients and 4 h following a high-fat meal (T_{max}) [17] and subsequently follows a biphasic elimination [22] with a terminal half-life of 5–16 h [17, 18, 22]. Further metabolic reactions generate its two main but inactive circulating metabolites, abiraterone sulphate and N-oxide abiraterone sulphate. The main metabolite excreted in urine is N-oxide abiraterone sulfate. Faeces is the primary route of excretion (87.9 %), with major components unchanged AA (55.3 %) and abiraterone (22.3 %) [23, 26]. Interestingly, the apparent clearance of Abiraterone is lower in CRPC patients compared to healthy subjects [23].

There is a significant inter-subject and within-subject variability regarding C_{max} and drug exposure, as measured with the area under the concentration-time curve (AUC) of approximately 40–70 % [22, 23]. There is also variability between cycles, with the C_{max} observed in cycle 1 being higher by 10–15 % than the C_{max} at later

Table 9.1 Phase II studies of AA in CRPC

Phase II studies of abiraterone acetate in CRPC							
Patients	Chemotherapy setting	Prior ketoconazole (%)	PSA ≥50 % (%)	ORR (%, evaluable patients)	Time to PSA progression, median days (95 % CI)	Population	First author (year of publication)
42	Naïve	2 (4.8 %)	28 (67 %)	9/24 (37.5 %)	225 (162–287)	caucasian	Attard (2009) [19]
33	Naïve	0	26 (79 %)	9/13 (69 %)	496 (280–NE)	caucasian	Ryan (2011) [41]
48	Naïve	NR	30 (63 %)	4/18 (22 %)	NE[a]	asian	Matsubara (2014) [27]
47	Pretreated	8 (17 %)	29 (51 %)	8/30 (27 %)	169 (113–281)	caucasian	Reid (2010) [20]
58	Pretreated	27 (46.6 %)	22 (36 %)	4/22 (18 %)	169 (82–200)	caucasian	Danila (2010) [21]
46	Pretreated	0	16 (35 %)	1/22 (4 %)	142 (85–NE)	asian	Satoh (2014) [28]
35	Pretreated	0	35 (43 %)	2/50 (4 %)	143 (113–252)	asian	Kwak (2014)

[a]Median time not reached
Abbreviations: *NE* not estimable; *NR* not reported

cycles [17, 18]. AUC and C_{max} increase with incremental dose proportionally between 750 and 1000 mg but more than proportionally between 500 and 750 mg [17, 22]. When administered with high-fat content food, drug exposure is increased by 4.4-fold compared to fasting achieved by extended absorption without any observed increase of C_{max} [17, 18]. Beyond food intake and cancer status, other covariates such as age, prior chemotherapy status, testosterone level, body mass index and total plasma proteins were not found to impact the PK of Abiraterone [23].

Pharmacodynamics

The changes of hormone levels of the steroidogenesis pathway upstream and downstream of CYP17 (Fig. 9.3) served as pharmacodynamic markers for the phase I trials. Both testosterone and androstenedione were suppressed to undetectable levels (<1 ng/dL) at all Abiraterone dose levels already by day eight for the study by Attard et al. and by day 28 in Ryan et al. (Fig. 9.4) [17]. By day 28, DHEA was decreased by a median of 3.4-fold but was still at detectable levels in contrast to the other androgens; this was caused by interference of DHEA with the used assay. ACTH was increased up to six-fold, as well as the levels of steroid precursors upstream of CYP17A1, namely deoxycorticosterone (median 10-fold) and corticosterone (median 40-fold) with a plateau of endocrine effects observed at the dose level of 750 mg. Administration of dexamethasone, given at PSA progression, resulted in suppression of ACTH and decrease of upstream steroids. The recommended phase II dose was selected at 1000 mg once daily based on these PD endocrine studies [17]. The phase I study by Ryan et al. confirmed the aforementioned endocrine changes and concluded to the same recommended dose for the phase II [29].

Drug Interactions

AA has been shown to be a strong inhibitor of CYP1A2 and CYP2D6 and a moderate inhibitor of CYP2C9, CYP2C19, CYP3A4 and CYP3A5 in vitro [30]. A phase I drug-drug interaction (DDI) study in CRPC indicated only a relevant in vivo interaction between AA-P and CYP2D6, but not with CYP1A2, after single dose co-administration with dextromethorphan, a CYP2D6 substrate, and theophylline, a CYP1A2 substrate [31]. An in vitro study indicated Abiraterone could decrease CYP3A4-dependent hydroxylation of its substrates 1α,25-dihydroxyvitamin D3 (calcitriol, active vitamin D) and midazolam [32]. In view of the reported in vivo interactions caution especially is indicated with concomitant administration of AA with known CYP2D6 substrates.

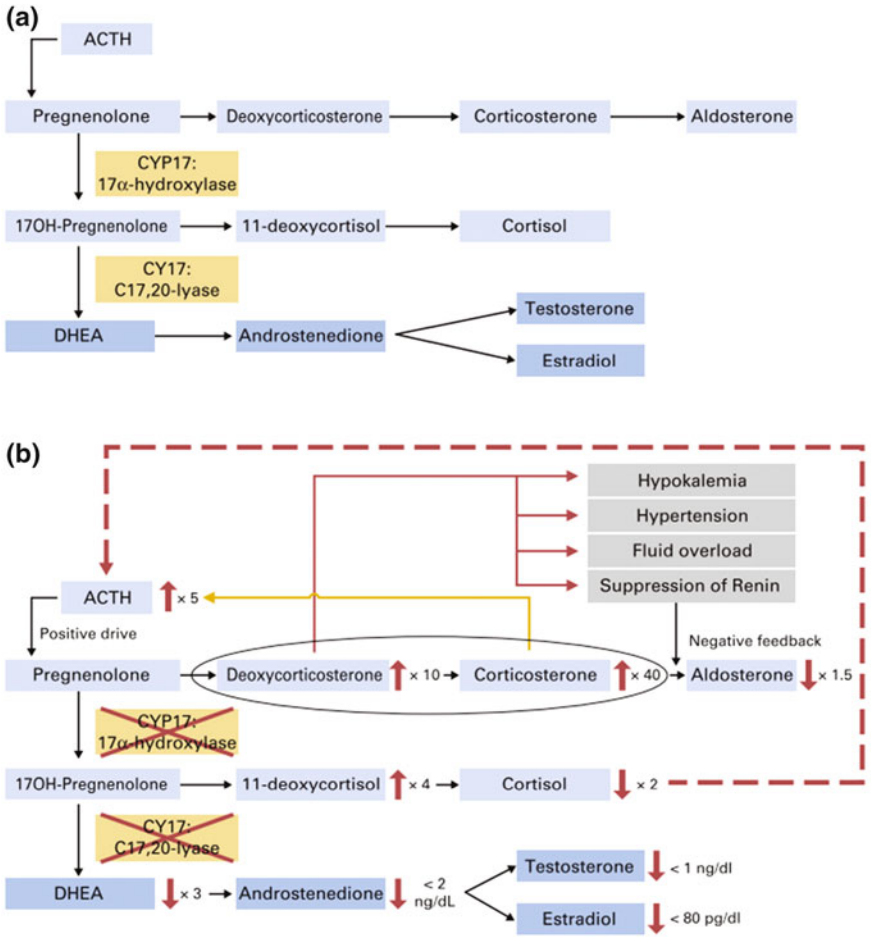

Fig. 9.3 CYP17 has 17a-hydroxylase and C17,20-lyase activity necessary for the conversion of 21-carbon pregnanes into 19-carbon sex steroid precursors. **a** Androgen biosynthesis pathway. **b** Abiraterone inhibits 17a-hydroxylase resulting in feedback increase in ACTH with subsequent increase in deoxycorticosterone and corticosterone and symptoms of mineralocorticoid excess. Inhibition of C17,20-lysase results in suppression of DHEA, androstenedione and testosterone. Adapted from Attard et al. [17]. With permission from American Society of Clinical Oncology

Mechanisms of Action of Abiraterone

Abiraterone blocks the CYP17A1 17α-hydroxylase/17,20-lyase enzyme via formation of a covalent bond between the nitrogen of its pyridine group and the haem iron of CYP17 (Fig. 9.5a,b) while a hydrogen bond network contributes to the interaction [33]. Inhibition of the hydroxylase activity suppresses hydroxylation of pregnenolone and progesterone at C17 whereas inhibition of the lyase activity limits

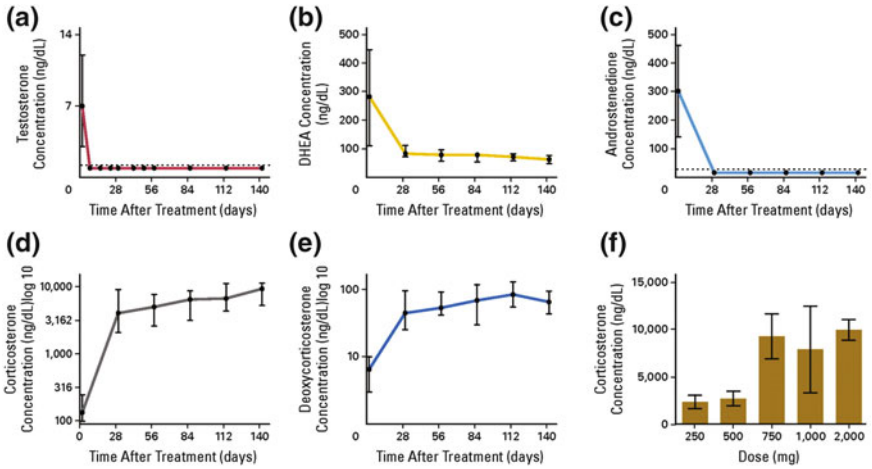

Fig. 9.4 Pharmacodynamic end points of the treatment with single agent Abiraterone Acetate. Treatment with Abiraterone Acetate results in significant suppression of testosterone, dehydroepiandrostenedione (DHEA), and androstenedione (**a**–**c**) and an increase of corticosterone and deoxycorticosterone (**d**–**e**). Plateau of endocrine effect after the dose of 750 mg (**f**). Adapted from Attard et al. [17]. With permission from American Society of Clinical Oncology

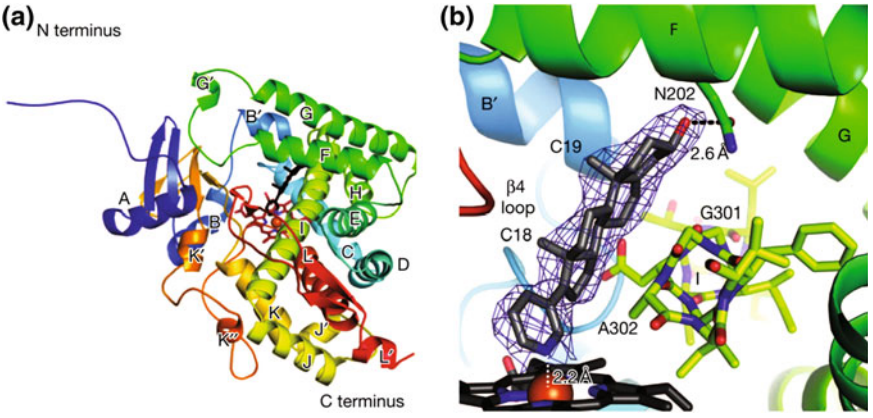

Fig. 9.5 Representation of CYP17A1-Abiraterone bound structure. **a** CYP17A1 from the N terminus (*blue*) to the C terminus (*red*). **b** Abiraterone binds at an angle of approximately 60° from haem against helix I (*yellow*). Adapted from DeVore and Scott [33]. With permission from Nature Publishing Group

the subsequent conversion of the hydroxylated metabolites to dehydroepiandrosterone and androstenedione, respectively, resulting in decreased testosterone and DHT levels (Fig. 9.3a, b). In addition to its 17α-hydroxylase/C17,20-lyase

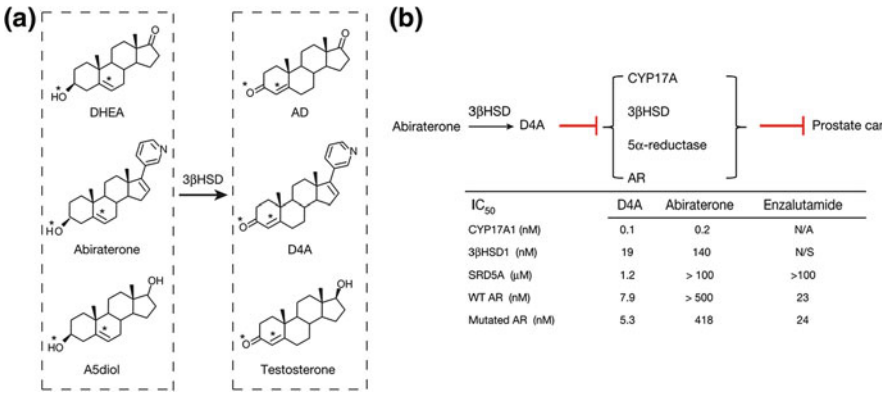

Fig. 9.6 D4A, an Abiraterone metabolite, has the ability to interact with multiple enzymes of the androgen biosynthesis pathway. **a** Conversion from Abiraterone to D4A by 3ßHSD. **b** D4A IC_{50} inhibitory activity in comparison to Abiraterone and Enzalutamide. Abbreviations: *AD* androstenedione; *A5diol* Δ5-androstenediol; *D4A* Δ^4-Abiraterone. Adapted from Li et al. [36]. With permission from Nature Publishing Group

inhibiting activity, Abiraterone also was shown to block 3-hydroxysteroid dehydrogenase (3ßHSD) [34] which transforms DHEA to androstenedione. Abiraterone can also interact with the AR directly acting in vitro as an antagonist of both the wild type and mutant AR but at the dose of 5 μM which is not achieved in plasma under normal administration [35]. It was also found to undergo a 3ßHSD enzymatic conversion into Δ^4-Abiraterone, in which the double bond is moved from C5 to C4 (D4A; Figs. 9.2, 9.6a) [36]. D4A shares identical steroid A and B rings with testosterone, enabling a strong antagonistic interaction with wildtype and mutant AR. D4A also inhibits both 3ßHSD and 5α-reductases (SRD5A) (Fig. 9.6b) while it retains CYP171A inhibitory activity comparable to Abiraterone.

Mineralocorticoids are able to inhibit AR transcriptional activity *in vitro* in the presence of androgens, at concentrations similar to those measured in Abiraterone-treated patients [37]. In the phase III studies a significant proportion of patients experienced symptoms associated with mineralocorticoid overload, and it is not inconceivable, that in this group of patients the mineralocorticoid antagonistic effects on AR could contribute to Abiraterone's clinical activity.

Efficacy of Abiraterone

After the promising results of the phase I and II trials AA was further evaluated in the phase III trials COU-AA-301 and COU-AA-302 in patients with mCRPC who had progressed on docetaxel in the former and were chemotherapy naive in the latter. In both trials patients were blindly randomized between the combination of AA 1000 mg once a day with prednisone 5 mg twice a day in the experimental arm —from now on in this work designated as AAP—and placebo plus prednisone

5 mg twice a day in the control arm respectively—from now on designated as PP. Inclusion criteria, randomization scheme, numbers of patients and stratification factors are summarized in Table 9.2.

The first interim analysis of COU-AA-301 was announced after 552 events and a median follow-up of 12.8 months (m) and showed an absolute improvement in the median Overall Survival (mOS) of 3.9 m in AAP compared to PP which remained significant after adjusting for stratification factors [38]. Subsequently the trial was un-blinded, crossover of patients from placebo to active drug was allowed and the drug gained regulatory approval for mCRPC patients who progressed after treatment with docetaxel. In the final survival analysis performed with the data available before crossover [39], after a median follow up of 20.2 months and 775 death events both the primary and secondary endpoints remained in favour of the AAP arm with an absolute benefit of 4.6 m in mOS (Table 9.3; Fig. 9.7). In multivariate analysis all the pre-specified stratification factors proved to be prognostic for survival and AAP showed superior survival compared to PP in all subgroups, while in some of them it did not reach statistical significance probably due to their small size (Fig. 9.8).

The COU-AA-302 trial enrolled chemo-naive patients with good prognostic parameters (Table 9.2) i.e. absence of significant pain, liver disease, low haemoglobin or albumin. The primary endpoints of the trial were radiographic PFS and OS and clinically meaningful secondary endpoints were captured: time to cytotoxic chemotherapy initiation, time to opiate use for cancer related pain, time to prostate specific antigen progression and time to performance status deterioration.

Two interim analyses (IA) were published on this trial and neither could demonstrate a survival benefit consistent with the pre-specified criterion for efficacy [40, 41]. This was subsequently met in the final overall analysis [42]. The first IA was performed after 43 % of the death events and showed a beneficial effect of AAP in the risk for radiographic progression or death with a hazard ratio of 0.49 as well as in the risk for death with a HR of 0.75[41] (Table 9.5). All clinically meaningful secondary endpoints-median time to cytotoxic chemotherapy, median TTPP, median time to performance status deterioration by 1 grade [41] (Table 9.4)-favoured AAP despite the fact that the median OS of 27.2 m reported in the PP group was the largest ever reported in that patient population. As a result the study was un-blinded and crossover allowed and AA's regulatory approval in the US and Europe was expanded to the pre-chemotherapy setting. The second published IA was performed shortly after the un-blinding of the study when only 3 patients had crossed over to active treatment from placebo. Again AAP showed an improved trend in OS compared to PP and an impressively improved time to rPFS of 16.5 m compared to 8.2 m of PP and this favourable effect was consistent in all stratification subgroups irrespective of age, baseline PSA, serum LDH or ALP, ECOG PS, presence or not at entry of pain or bone metastases [40]. In the final overall analysis after 49.2 months of follow up, 65 % of patients had died in AAP compared to 71 % in PP [42]. Only 67 % of the patients in the Abiraterone group compared to 80 % of patients in the placebo group had received further treatments. The final analysis showed increased mOS in the Abiraterone group of 34.7 months versus 30.3 months in the placebo group which met the pre-specified statistical

Table 9.2 Inclusion criteria and stratification factors in COU-AA-301 and COU-AA-302

	COU-AA-301	COU-AA-302
Number of patients	1195	1088
Randomization ratio	2:1	1:1
Inclusion criteria		
Prior treatment	Post docetaxel	Pre docetaxel
ECOG PS	≤2	≤1
ALT and AST	<2.5 times ULN or < 5 times the ULN if liver lesions present	<2.5 times ULN
Albumin	>3 g/dL	>3.5 g/dL
Haemoglobin	≥9.0 g/dL	≥10.0 g/dL
Platelet Count	≥100,000/μL	≥100,000/μL
Visceral disease	Allowed	Not allowed
Score of BPI-SF question 3	Any	0–3
Type of progression to prior treatment	Radiologic or PSA progression	Radiologic or PSA progression
Primary endpoints		
	Overall survival	Overall survival
		Radiologic progression free survival
Secondary endpoints		
	Time to PSA progression	Time to PSA progression (TTPP)
	Radiologic progression free survival	
	PSA response rate	
		Time to opiate use for cancer-related pain
		Time to initiation of cytotoxic chemotherapy
		Time to ECOG performance status deterioration
Stratification factors		
	ECOG performance status 0–1 versus 2	ECOG performance status 0 versus 1
	Presence of significant pain in the past 24 h before randomization as captured by the Brief Pain Inventory-Short Form (BPI-SF) question 3 (Yes vs. No)	
	Number of previous chemotherapy regimens (1 vs. 2)	
	PSA progression only versus radiographic progression regardless of PSA progression	

Table 9.3 Outcomes of efficacy endpoints in COU-AA-301

COU-AA-301	COU-AA-301 1st interim analysis [38]			COU-AA-301 final analysis [39]		
	AAP	PP	HR [95 % CI]	AAP	PP	HR [95 % CI]
Duration of drug exposure (months)	8	4		7.4	3.6	
Median overall survival (months)	14.8	10.9	0.65 [0.54–0.77]	15.8	11.2	0.74[0.64–0.86]
Time to PSA progression (months)	10.2	6.6	0.58; [0.46–0.73]	8.5	6.6	0.63[0.52–0.78]
rPFS (months)	5.6	3.6	0.67[0.58–0.78]	5.6	3.6	0.66[0.58–0.76]
PSA response rate	29 %	6 %		29.5 %	5.5 %	
Response rate by RECIST in patients with measurable disease	14 %	3 %		14.8 %	3.3 %	

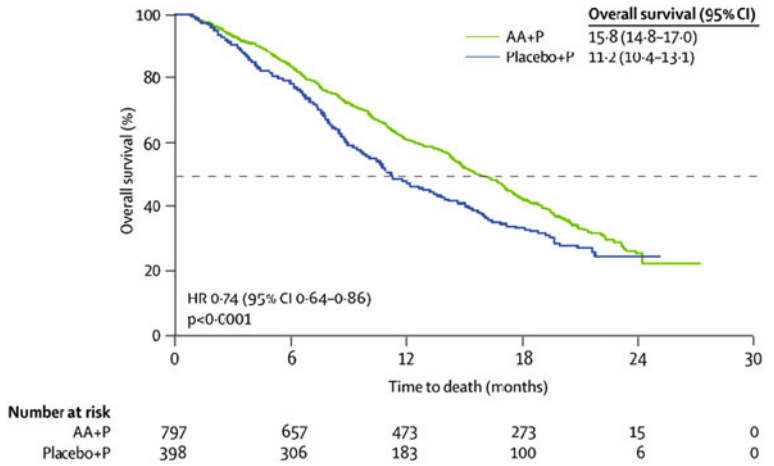

Fig. 9.7 Overall survival in the COU-AA-301 trial as evaluated in the last analysis at the time point of crossover. Adapted from Fizazi et al. [39]. With permission from Elsevier

boundary for efficacy. The radiographic progression free survival endpoint was also met by the trial [42] (Table 9.5).

It is noteworthy that the Kaplan-Meier curves in COU-AA-302 started separating at 12 months and most clearly so after 18 months [42] which also explains why there was no significant survival benefit evident between AAP and P in the interim analyses (Fig. 9.9). This can be attributed to the inclusion of patients with good prognosis and the resulting small number of death events in the beginning of the trial. Still, during the first 12 months of the trial approximately 10 % of the patients died and 25–30 % of these deaths could be attributed to non cancer-related

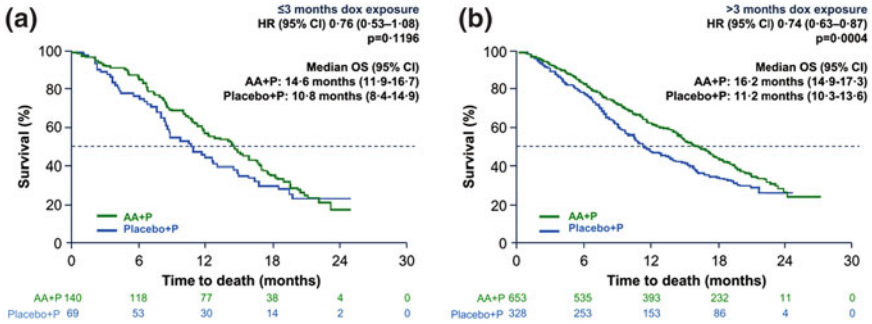

Fig. 9.8 Overall survival in COU-AA-301 based on duration of previous docetaxel chemotherapy (**a** ≤3 months and **b** >3 months). The group of ≤3 months seems to have a non-statistically significant HR of 0.76 (95 % CI 0.53–1.08) and could be considered as not deriving any benefit from treatment with Abiraterone. Nevertheless, the 209 patients represented in this group are substantially less compared to the 981 of the >3 months group. Additionally, the survival curves appear to have a divergent course. Adapted from Fizazi et al. [39]. With permission from Elsevier

Table 9.4 Outcomes of secondary endpoints in COU-AA-301 and COU-AA-302

	AAP [95 % CI] (months)	PP [95 % CI]
COU-AA-301		
Improvement of fatigue intensity (%) [43]	58.1	40.3
Improvement of fatigue interference (%) [43]	55	38
Median time to fatigue improvement (days) [43]	59	194
Time to fatigue intensity progression [25th percentile] (days) [43]	232	139
Pain palliation (%) [44]	45	28.8
Median time to palliation of pain intensity (months) [44]	5.6	13.7
Median duration of palliation of pain intensity (months) [44]	4.2	2.1
Median time to occurrence of first skeletal related event (months) [44]2	25	20.3
Improvement in the FACT-P total score (%) [46]	48	32
Median time to deterioration of FACT-P(weeks) [46]	59.9	36.1
COU-AA-302		
Median time to cytotoxic chemotherapy [40]	26.5	16.8
Median time to decline in ECOG PS	12.3	10.9
Pain		
Median time to opiate use (months) [40]	33.4	23.4
Median time to pain progression (months) [41]	26.7 [19.3–not estimable]	18.4 [14.9–not estimable]
Median time to progression of the pain interference with daily activities (months) [45]	(10.3 [9.3–13.0]	7.4 [6.4–8.6]

(continued)

Table 9.4 (continued)

	AAP [95 % CI] (months)	PP [95 % CI]
Median time to progression of the worst pain (months) [45]	26.7 [19.4–not estimable]	(19.4 [16.6–not estimable])
QOL		
Median time to deterioration of QOL (as measured by FACT-P) (months) [45]	12.7 [11.1–14.0]	8.3 [7.4–10.6]
Median time to deterioration of QOL (as measured by PCa specific FACT-P subscale) (months) [45]	11.1 [8.6–13.8]	5.8 [5.5–8.3]

Table 9.5 Outcomes of efficacy endpoints in COU-AA-302

	COU-AA-302 1st interim analysis [41]			COU-AA-302 2nd interim analysis [40]			COU-AA-302 final analysis [42]		
	AAP	PP	HR [95 % CI]	AAP	PP	HR [95 % CI]	AAP	PP	HR [95 % CI]
Median overall survival (months)	Not reached	27.2	0.75 [0.61–0.93]	35.3	30.1	0.79 [0.66–0.95]	34.7	30.3	0.81 [95 % CI 0 ·70–0 · 93]
Time to PSA progression (months)	11.1	5.6	0.49 [0.42–0.57]	NR	NR	NR	NR	NR	NR
rPFS (months)	16.5	8.3	0.53 [0.45–0.62]	16.5	8.2	0.52 [0.45–0.61]	NR	NR	NR
PSA response rate	62 %	24 %		68 %	29 %		NR	NR	NR
Response rate by RECIST in patients with measurable disease	36 %	16 %		NR	NR		NR	NR	

NR not reported

morbidity [42]. The rest of the patients who died in this time period, approximately 7 % of the total patients, potentially reflect patients with disease of bad prognosis that was not captured by the prognostic factors applied in the inclusion criteria and who did not respond either to Abiraterone or to prednisone.

Effect of Abiraterone on Secondary Endpoints

In both COU-AA-301 and COU-AA-302 trials secondary endpoints were captured to substantiate the clinical effect of Abiraterone and all of them favoured AAP over PP (Table 9.4).

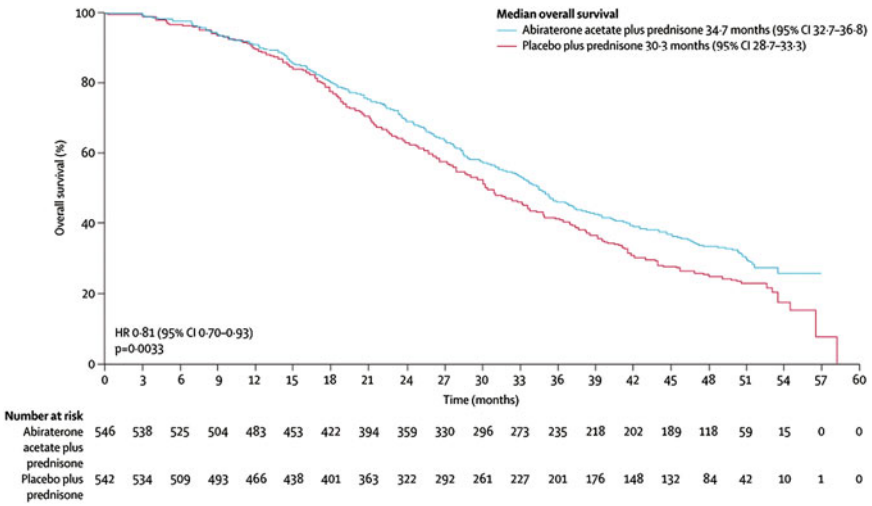

Fig. 9.9 Overall survival curves in COU-AA-302. Noteworthy is the fact that the curves started separating only after 12–18 months of treatment. Adapted from Ryan et al. [42]. With permission from Elsevier

Fatigue

In COU-AA-301 fatigue was evaluated at each cycle of treatment by assessment of the Brief Fatigue Inventory questionnaire which evaluates fatigue intensity and fatigue interference with aspects of everyday life. Improvement of fatigue intensity and fatigue interference was significantly higher in AAP compared to PP (58.1 % vs. 40.3 % and 55 % vs. 38 % respectively). Additionally, in the AAP arm the time to improvement of fatigue was substantially shorter and the time to fatigue progression delayed compared to the PP arm [43]. In COU-AA-302 fatigue was not prospectively captured.

Pain Palliation and Progression

Pain was evaluated throughout COU-AA-301 and COU-AA-302 by means of the BPI-SF. Pain intensity was defined as the score of the BPI-SF question 3 (worst pain in the previous 24 h) and pain interference was defined as the mean of all seven questions assessing pain interference with everyday activities on the BPI-SF. Additionally, in COU-AA-301 the mean analgesic use was scored as per WHO criteria. In a post hoc analysis of COU-AA-301 with the data available at the time-point before the crossover to active drug the median time to pain progression was not reached at that time point, yet significantly more patients in the AAP group had experienced pain palliation and improvement of pain interference than in the PP group and pain palliation occurred faster in AAP (Table 9.4) [44].

In COU-AA-302 patients did not have significant pain at treatment initiation but AA delayed pain occurrence compared to placebo as indicated by the delayed time to all pain related parameters: median time to pain progression, median time to opiate use [41, 42], median time to progression of the pain interference with daily activities and the median time to progression of the worst pain [45] (Table 9.4).

Skeletal Related Events

In COU-AA-301 the proportion of patients with skeletal related events was similar in both groups but there was a significantly longer median time to skeletal related events in Abiraterone (25 m) compared to placebo (20.3 m)(Table 9.4) [44]. Skeletal related events were not captured in COU-AA-302.

Quality of Life

In COU-AA-301 and 302 health related quality of life (HRQoL) was assessed by means of the Functional Assessment of Cancer Therapy-Prostate (FACT-P) questionnaire which has subscales to assess different aspects of QoL. In COU-AA-301, AA improved quality of life in more patients (48 % vs. 32 %) and in a shorter time compared to prednisone and delayed functional status deterioration by 6 months despite the fact that prednisone was able to produce substantial improvements in QOL as well [46]. In the minimally symptomatic population of COU-AA-302, AA delayed median time to deterioration of quality of life both as measured by the FACT-P total score and by the prostate-cancer-specific subscale [45] (Table 9.4).

Toxicity of Abiraterone

AA is a well tolerated drug as indicated by the fact that in COU-AA-301 the frequency of adverse events (AE) leading to treatment discontinuation was lower in AAP compared to PP (19 % vs. 23 %) [38] and in COU-AA-302 [42] the same frequency was again at the level of 19 % for AAP but only 12 % for PP [41], the latter reflecting a population with less advanced and symptomatic disease.

The most frequent adverse events in both trials were fatigue, back pain, constipation, diarrhoea, nausea, bone pain and arthralgia. These occurred in similar frequency in the experimental and control groups [38, 39, 41, 42] and constitute disease related symptoms rather than Abiraterone related AE (Table 9.6). A contribution of Abiraterone to arthralgia, fatigue and diarrhoea cannot be excluded as these AEs showed consistently a mildly increased frequency in both trials in the AAP over the PP arm (Table 9.6).

Table 9.6 Adverse events grade 1–4 in AA phase III trials

Adverse event	COU-AA-301		COU-AA-302	
	AAP (%)	PP (%)	AAP (%)	PP (%)
Anaemia	25	28	NR	NR
Fatigue	47	44	39	34
Back pain	33	36	32	32
Arthralgia	30	24	28	24
Nausea	33	33	22	22
Vomiting	24	26	NR	NR
Constipation	28	32	23	19
Hot flush	NR	NR	22	18
Diarrhoea	20	15	22	18
Bone pain	27	30	20	19
Pain in extremity	20	21	17	16
Cough	NR	NR	17	14
Urinary tract infection	13	7	2	<1

Abbreviations: *NR* not reported
Based on data from Refs. [38, 41, 42]

On the other hand, AEs related to mineralocorticoid excess (ME) (hypokalemia, fluid retention, hypertension) were more frequent in AAP compared to PP in both trials [38, 42] (Table 9.7). ME observed in CRPC patients treated with single agent AA can be managed either by the addition of eplerenone, a mineralocorticoid receptor antagonist or by exogenous corticosteroids [17, 47] that inhibit secretion of corticotrophin-releasing hormone and ACTH, therefore exogenous corticosteroids are routinely co-administered with Abiraterone. Use of spironolactone for the management of ME should be avoided as it can act as an agonist for wildtype AR [35, 48]. Hypertension that arises despite steroid use can be managed symptomatically by addition of an antihypertensive agent but diuretics should be avoided as they may exacerbate Abiraterone induced hypokalemia. Hypokalemia can be treated by supplementation with potassium.

Cardiac events were consistently more frequent in AAP rather than in PP in both trials although this difference did not reach statistical significance. Most frequent were grade 1 or 2 tachycardia and grade 3 or less atrial fibrillation [39] and fatal cardiac events were rare and approximately 1 % for each arm in both trials [38, 42] (Table 9.7). No effect of AA could be detected on the QTcF and QRS interval by a further small study evaluating ECG changes with AA [49].

Early in the course of the COU-AA-301 trial an incident of grade 4 liver function test (LFT) elevation instigated patients' visits to be performed biweekly during the first 12 weeks for close follow up of LFTs; in this way hepatotoxicity was recognized and treated early by treatment interruption when LFTs increase was grade 3 or more and Abiraterone was reinitiated at a lower dose after normalization. At the

Table 9.7 Toxicities of special interest in COU-AA-301 and COU-AA-302

	COU-AA-301				COU-AA-302				Open label expansion cohort
	All grades		Grade 3–4		Grade 1–2		Grade 3–4		Grade 3–4
	AAP (%)	PP (%)	AAP (%)	PP (%)	AAP (%)	PP (%)	AAP (%)	PP (%)	AAP (%)
Hypokalemia	17	8	<4	<1	16	11	<3	2	1
Hypertension	10	8	1	<1	19	11	5	3	4
Fluid retention	31	22	<3	1	30	23	1	<2	1
Cardiac disorders	13	11	4	<3	15	14	7	<4	2
Atrial fibrillation	2	1	NA	NA	4	4	<2	0	NA
ALT increase[a]	10	8	<4	<4	7	4	<6	<2	8
AST increase[a]					9	4	3	<1	

[a]In COU-AA-301 liver function abnormalities were grouped together as "LFT abnormalities"
Based on data from Refs. [38, 42, 50]

end LFT elevation frequency was similar in COU-AA-301 between the placebo and control arm. Still in COU-AA-302, though the same schedule of visits was maintained, ALT and AST elevations were more common in AAP [42] (Table 9.6 and 9.7).

Urinary tract infections were significantly more frequent in the AAP arm (12 % vs. 7 %) in COU-AA-301 [38] whereas in COU-AA-302 this finding was not confirmed [41] and its significance is questionable. No new safety signals were detected in the open label early-access protocol launched after publication of the COU-AA-301 [50].

Special Groups of Patients

Patients with Visceral Disease

Patients with visceral disease represent approximately 5–24 % of mCRPC patients and are considered to have worse prognosis [51]. COU-AA-301 allowed participation of patients with visceral disease while COU-AA-302 did not. Patients with visceral disease showed improved survival with Abiraterone compared to prednisone in the post-chemotherapy setting (HR 0.70;95 % CI [0.52–0.94]) which reached statistical significance in the first interim analysis before crossover was allowed [38] but not in the final survival analysis while it still maintained a favourable trend (HR 0.79; 95 % CI [0.59–1.05]) [39]. In a separate exploratory analysis of the visceral disease cohort [52] the secondary endpoints of rPFS, PSA

response rate (28 % vs. 7 % in the visceral disease subset and 30 % vs. 5 % in those without visceral disease) and objective response rate showed similar benefit in the subgroups with and without visceral disease from the treatment with Abiraterone. AEs and especially LFT derangement, fluid retention and hypokalemia were similar in both groups.

Patients with Pain

Presence of pain and especially the use of opioid analgesics is an established adverse prognostic factor in mCRPC patients. The COU-AA-301 trial enrolled patients regardless of the presence of pain but COU-AA-302 excluded patients with significant pain or on opioid analgesics. In COU-AA-301 patients with significant pain, defined as a score of 4 or more on a 0 to 10 scale rating the worst pain during the last 24 h, derived significant benefit from the treatment with Abiraterone. The HR for death in the subgroups with and without significant pain were almost identical −0.68 [95 % CI 0.53–0.85] and 0.64 [95 % CI 0.50–0.82] respectively—in the first interim analysis [38] and was maintained also in the final analysis after the crossover—HRs of 0.78 [95 % CI 0.63–0.96] and 0.69 [95 % CI 0.56–0.85] respectively [39].

Elderly Patients

Elderly patients, i.e. with an age of ≥75, were well represented in both COU-AA-301 and COU-AA-302 with 331 patients in the former and 350 in the latter. Abiraterone was effective in elderly patients in both COU-AA-301 [53] and COU-AA-302 [54] with similar HRs to the younger patients despite the latter living longer [54]. Additionally, TTPP and rPFS as well as PSA response rates were in favour of the Abiraterone arm for the elderly as well as for the younger group in both trials [53] and secondary endpoints like time to initiation of chemotherapy, time to opiate use, and time to ECOG PS deterioration substantiated the benefit the elderly derived from the treatment with AAP [54].

The AE profile was similar in the two age groups following the trend of the general trial population and is summarized in Tables 9.8 and 9.9 with fatigue being the most common AE in both.

In COU-AA-301 Grade 3/4 AEs were of similar frequency in both age groups: 62 % in the elderly and 60 % in younger patients. Comparably, in COU-AA-302 the frequency of Grade 3/4 AEs seemed to be lower altogether but strongly age related: 40 % for patients younger than 65 years, 48 % for patients 65 to 74 years old and 57 % for older than 75 years.

Consistently with the above, discontinuation due to AEs in COU-AA-301 was equally frequent in the two age groups in both arms and more frequent than in COU-AA-302, but in the latter the elderly experienced more often discontinuation

Table 9.8 AEs in elderly patients (≥75 years) compared to younger ones (<75 years) in the COU-AA-302 trial

	Elderly		Younger	
	AAP	PP	AAP	PP
Median exposure	11.8 m	8.5 m	14.4 m	8.2 m
More than one dose reduction	12 %	4 %	4 %	3 %
Rate of discontinuation due to adverse events	15 %	10 %	5 %	4 %
Fatigue (All Grades)	42 %	38 %	39 %	33 %
Grade 3–4 fatigue	6 %	4 %	<1 %	<1 %
AEs of special interest	74 %	59 %	66 %	48 %
Peripheral Oedema	35 %	32 %	22 %	16 %
Grade 1–4 hypokalemia	17 %	10 %	18 %	14 %
Grade 3–4 hypokalemia	4 %	3 %	2 %	1 %
Grade 1–4 hepatotoxicity	21 %	15 %	18 %	10 %
Grade 3–4 hepatotoxicity	8 %	4 %	8 %	2 %
Grade 3–4 cardiotoxicity	9 %	5 %	6 %	3 %
Grade 3–4 hypertension	5 %	6 %	4 %	2 %
Treatment emergent AES leading to death	8 %	6 %	2 %	2 %

Based on data from Ref. [54]

Table 9.9 Tolerability of treatment in COU-AA-301 and COU-AA-302 for younger and elderly patients

	COU-AA-301				COU-AA-302			
	Elderly		Younger		Elderly		Younger	
Grade 3/4 AES	62 %		60 %		57 %[a]		48 %; 40 %[a]	
Treatment arm	AAP	PP	AAP	PP	AAP	PP	AAP	PP
Median exposure	8.9 m	3.6 m	7.9 m	3.9 m	11.8 m	8.5 m	14.4 m	8.2 m
More than one dose interruption	27%	22%	18%	15%	12 %	4 %	4 %	3 %
More than one dose reduction	<3 %				12 %	4 %	4 %	3 %
Rate of discontinuation due to adverse events	15 %	18 %	13 %	18 %	15 %	10 %	5 %	4 %

[a]Refers to the age groups: >74, 65–74, <65 respectively

due to an AE and more so in the AAP arm. In a similar way, the incidence of dose interruptions was higher in the COU-AA-301 and lower in COU-AA-302 with the highest frequency observed in both trials in the elderly in the AAP group (Table 9.9). Finally, the frequency of dose reductions was similar in all subgroups in both arms and around 3–4 % with only exception the elderly in AAP of COU-AA-302 with 12 %.

Although hypokalemia was equally frequent in both age groups in both trials the other ME related AEs-hypertension and edema—were marginally more frequent in

the elderly (Table 9.8). Cardiac events were also more frequently increased in AAP compared to PP in the elderly (atrial fibrillation, tachycardia) but significant cardiac events leading to study discontinuation or cardiac death were rare and of similar frequency in both age groups in both arms [53].

In conclusion, AAP was mildly less well tolerated in the elderly and this was more pronounced in the pre-chemotherapy setting probably due to longer exposure while the differences of elderly to younger patients in the post-chemotherapy setting were less significant potentially due to shorter exposure or due to the increased weight of disease related symptoms.

Patients with Poor Performance Status

Patients with a performance status of 2 were excluded from the pre-chemotherapy COU-AA-302 trial while a PS of 2 was allowed in the post-chemotherapy COU-AA-301 trial. In the final analysis, the benefit of this group from AA although exhibiting favourable trend, did not reach statistical significance and there was only a small absolute median survival difference of 0.3 months between the two arms [39]. Still, the survival curves for patients with PS of 2 in COU-AA-301 seem to largely overlap until 6 months after initiation of treatment and only then do they start to split; by that time almost half of the patients have died and any benefit becomes visible thereafter (Fig. 9.10). Certainly, this post hoc analysis with a total

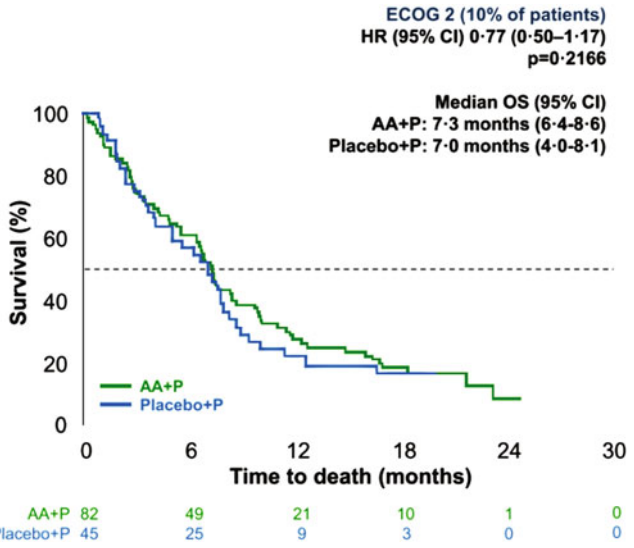

Fig. 9.10 Survival curves for the subgroup of patients with PS of 2 in COU-AA-301. Only 127 patients fall within this subgroup providing only limited statistical power. The survival curves are largely overlapping until 6 months after initiation of treatment when almost half of all patients in each arm have died. Subsequently the curves start splitting with a small benefit for the AAP arm becoming visible. Adapted from Fizazi et al. [39]. With permission from Elsevier

number of 127 patients was not sufficiently powered to detect a small difference if present. Additionally other available treatments for mCRPC are not substantially active in this subgroup either [55, 56] and in view of the favourable toxicity profile AA should be considered as an option for these patients.

Abiraterone and Steroids

AA is routinely administered with prednisolone or prednisone 5 mg BD to mitigate mineralocorticoid excess. In the initial phase I trial it was realized that addition of dexamethasone to monotherapy with Abiraterone after PSA progression can produce PSA responses in approximately 33 % of the patients even in patients pretreated with dexamethasone monotherapy [57]. Subsequently, it was also documented in a retrospective analysis that at PSA progression on the combination of Abiraterone and prednisone replacing prednisone 5 mg BD with dexamethasone 0.5 mg OD also results into ≥50 % PSA responses in 6 out of 30 patients. In 2 out of 9 patients with evaluable disease by RECIST at baseline a partial response could be documented after the steroid switch [58].

On the other hand steroids are potential drivers of PCa. They can act as activating ligands for promiscuous mutated androgen receptors e.g. bearing the L702H or T787A mutation [59] and also interact with the Glucocorticoid Receptor, known to drive the expression of AR-controlled genes under conditions of androgen blockade with enzalutamide [60]. Still, administration of Abiraterone monotherapy cannot be recommended due to the increased risk of toxicity while the optimal adjunct steroid and steroid schedule for the treatment with Abiraterone is being addressed in an ongoing trial (NCT01867710).

Abiraterone Pre and Post Enzalutamide

Abiraterone and Enzalutamide act on the same pathway in a different manner. Abiraterone's main mode of action is by is depleting the ligand while Enzalutamide is blocking the receptor and it is of no surprise that they exhibit a largely overlapping action and cross-resistance. In small retrospective studies patients progressing on Abiraterone when treated with Enzalutamide exhibited ≥50 % PSA declines in only 13–34 %, with median rPFS being reported between 2.8 and 6.6 m [62–66] while patients progressing on Enzalutamide, when treated with Abiraterone, had ≥30 % PSA response rates of approximately 10–18 % and median PFS of 3–4 m [67, 68].

Therefore, it does not seem to be beneficial for unselected patients progressing on one of the two agents to be exposed to the alternative one since it might potentially deprive them from the opportunity of having an active alternative treatment. Nevertheless, patients who do achieve PSA responses on sequential treatment have also prolonged PFS compared to those who do not and a small fraction not responding to prior Abiraterone treatment may respond to subsequent

Enzalutamide and vice versa [61]. Hence it is reasonable to try to identify these subgroups using predictive biomarkers and potentially attempt sequential treatment with Abiraterone and Enzalutamide in subgroups with good prognosis and increased chances of response.

Predictive Biomarkers

In COU-AA-301 and COU-AA-302 there was no clinical or laboratory parameter that could predict benefit from treatment with Abiraterone: regardless of age, presence of pain or visceral disease, levels of ALP, LDH and PSA below or above median treatment with Abiraterone was beneficial in all subgroups [38, 52]. Additionally, in contrast to other cancers, only rarely is there measurable disease present in mCRPC to allow for early evaluation of treatment's activity, therefore predictive biomarkers and validated early markers of treatment response are necessary to guide treatment decisions.

Gleason score at initial diagnosis of PCa, stratified as <8 or ≥8, did not have any predictive significance for the treatment with Abiraterone [69] and nor did the duration of previous androgen deprivation therapy [70]. Baseline serum levels of androgens collected prospectively during the COU-AA-301 trial proved to be rather prognostic than predictive as patients with serum androgen levels above the median had the longest OS. Still, Abiraterone was beneficial over prednisone at all levels of androgens and interestingly its effect was more pronounced in patients with lower androgens [71]. Increased nuclear IHC expression of AR and CYP17 expression of ≥10 % in samples obtained before administration of Abiraterone in a cohort of 25 patients, was found to correlate with longer time before discontinuation of Abiraterone acetate [72].

Serum PSA levels is an easily accessible biomarker and has been in use for a long time in mCRPC considered to reflect tumor burden; PSA velocity or doubling time has been associated with OS [73, 74], and PSA declines are routinely used as an adjunct in everyday clinical decision making and as an intermediate biomarker in clinical trials. In some of them, post-treatment PSA changes have been commonly found to correlate with OS but the utility of these changes as a surrogate biomarker for OS remains controversial as for example in the TROPIC trial a PSA response of ≥30 % was significantly associated with OS but not able to predict the full treatment effect, therefore failing the 3rd Prentice criterion [75]. Additionally, an initial surge of PSA before a subsequent decline is not infrequent after treatment with cytotoxics or even with Abiraterone and render PSA an inappropriate tool to decide on treatment discontinuation or not. Still, based on the COU-AA-301 and 302 data a number of PSA kinetic parameters, including ≥30 and ≥50 % PSA response rates for both chemotherapy pretreated and chemotherapy naive patients were found to be associated with OS and to meet the Prentice criteria for surrogacy [76].

Nevertheless in another study using data from COU-AA-301, PSA at week 12 whether reduced by 50 % or by 30 % was reported to have a lower predictive ability compared to CTCs in combination with LDH at week 12 which was also

shown to be a surrogate marker for survival, fulfilling the Prentice criteria for surrogacy [77]. Using these two biomarkers patients can be stratified to low risk (<5CTCs/7.5 ml) intermediate risk (≥5CTCs/7.5 ml and LDH ≤250u/l) and high risk (≥5CTCs/7.5 ml and LDH >250u/ml) with a two year survival of 46 %, 10 % and 2 % respectively.

Furthermore, genomic aberrations present in PCa have been also evaluated for their predictive value in the treatment with Abiraterone. *TMPRSS2*:*ERG* rearrangements detected in CTCs before initiation of Abiraterone [78] or in the primary biopsy tissue of patients participating in COU-AA-302 [79] did not have predictive value, though presence of more than one *ERG* fusion transcripts was associated with an impressive improvement in rPFS from 5.4 m with PP to 22.4 m with AAP [79]. PTEN loss, present in approximately 40 % of mCRPC patients [80], was found in a retrospective cohort of patients receiving Abiraterone in the post-docetaxel setting to be associated with a shorter mOS compared to PTEN expressing patients (14 m vs. 21 m, p = 0.004) but PSA response rate of >50 % was similar in both groups [81].

Patients with measurable CTCs at baseline expressing AR-V7, a ligand-independent splice variant of *AR*, did not respond to Enzalutamide or Abiraterone in contrast to those who were negative for AR-V7 and showed PSA response rates of 53 % for Enzalutamide and 68 % for Abiraterone. Evenmore, the predictive significance of AR-V7 was maintained after correcting for previous treatment with Abiraterone or Enzalutamide [82].

Plasma cell free DNA isolated from patients before and during treatment with Abiraterone can reveal *AR* copy number (CN) gains in 40 % of patients or *AR* point mutations (PM) in 17 % and these patients were found to be approximately 5 times less likely to have a ≥50 % PSA response and also had worse OS and PFS compared to patients with normal *AR* status [83, 84] while increased fraction of tumor circulating DNA was associated with worse OS and PFS. Interestingly, *AR*-CN gains and *AR*-PM were inversely associated with each other [83].

Potential Mechanisms of Resistance to Abiraterone

AR engagement with ligand and subsequent dimerization, escape from chaperones and nuclear translocation, all processes necessary for AR function [85] can be thought of, like any biological process, as a sequence of reversible reactions, with the equilibrium being shifted towards nuclear translocation when ligand is present. Potential mechanisms of resistance to Abiraterone have not yet been proven clinically but can be presumed based on the available clinical and preclinical data. They have to maintain AR transcriptional activity by shifting the equilibrium towards nuclear translocation in the absence of or despite the minimal amount of androgens and can be classified as follows.

Alternative Ligands for AR

Mutant AR (mAR) forms able to bind and become activated by exogenous or physiological ligands that are more abundant than the suppressed androgenic steroids have long been established. The T878A AR mutation can be activated by prednisolone and dexamethasone at the dose they are usually co-administered with Abiraterone [35] but also by progestagens and estrogens and first generation antiandrogens [86]. Cortisol and cortisone in physiological levels can activate AR with the double mutation L702H and T878A [87]. The L702H and T878A mutations, but not H875Y and T878S, were found to have consistently increased frequency in plasma DNA of patients progressing on Abiraterone compared to baseline and patients having these mutations at baseline are less likely to respond to Abiraterone [83]. Additionally, Abiraterone increases the levels of steroids upstream of CYP17 increasing availability of ligands for promiscuous non-specific binding [88].

Higher Affinity of AR for Physiologic Ligands

Abiraterone does not completely abrogate androgenic steroid synthesis and in the plasma and urine of mCRPC patients under Abiraterone treatment androgens are suppressed by about 95 % from their castrate levels and are still detectable with ultrasensitive methods [88]. Additionally, some androgens are produced through the alternative back-door pathway from 17-hydroxy-pregnenolone [88]. These residual androgens could maintain survival of PCa cells with mARs hypersensitive to AR concentrations in the femtomolar range [89].

Higher Concentration of AR

In human CRPC xenografts in mice Abiraterone treatment leads to increase of *AR* expression [90] and *AR* CN gains in circulating plasma DNA have been associated with reduced PSA response rates and smaller time to radiographic progression with Abiraterone treatment [83] indicating that overexpression of AR could be sufficient to induce resistance to Abiraterone acting in the absence of ligand potentially through mass-action, a mechanism well established in other receptors [91].

Alternative Splicing of AR

Ligand independent AR splice variants, able to maintain AR regulated gene expression in the absence of ligand, have been reported to be both more frequently present and more abundant after treatment with Abiraterone compared to baseline [90, 92–94] indicating a role in both primary and acquired resistance. Additionally,

AR-V7 expression in patients' CTCs seems to almost exclude a PSA response on treatment with either Abiraterone or Enzalutamide [82].

Alternative Receptors

Preclinical evidence suggest that GR could bypass AR during treatment with Enzalutamide by activating a subset of AR controlled genes; it therefore may represent a mechanism of resistance to Enzalutamide but it is unclear, whether GR could have the same role with Abiraterone [95].

Up-Regulation of CYP17

Another potential mechanism of resistance to Abiraterone is up-regulation of CYP17 and other enzymes of the androgen synthesis pathway as was observed in mice bearing PCa xenografts after exposure to Abiraterone [90]. This mechanism could offer an explanation for a report where patients who progress on Abiraterone can experience a PSA response after intake of Abiraterone with food [96]. Swallowing Abiraterone with food leads to increased bioavailability and potentially inhibition of more CYP17 molecules which until then might have been unaffected. Additionally, higher concentration of Abiraterone in the plasma could also inhibit alternative enzymatic targets such as the 3β-hydroxysteroid dehydrogenase who are considered to maintain residual androgen synthesis despite CYP17 inhibition by diverting steroids in the back-door pathway [97] and potentially also allow Abiraterone to act directly antagonistically on AR [35].

Synopsis and Prospects

Abiraterone is an effective treatment for mCRPC, well tolerated with beneficial effects in the quality of life of patients, able to control their symptoms. Still there are many unanswered questions. The most appropriate timing for its administration needs to be defined as well as its efficacy in the hormone naive setting or in locally advanced disease. Additionally it is not clear how it should be sequenced in respect to chemotherapy: before, after or in parallel? Answers to these questions are anticipated from ongoing active trials.

Finally, predictive biomarkers able to select for responders to Abiraterone are warranted especially in view of the appearance of new alternative agents. It would be also useful to predict which patients would benefit more from Abiraterone instead of Enzalutamide and vice versa. For these purposes, some first candidate biomarkers have been identified but they still need to be validated prospectively in larger patient populations. As new drugs and trials in mCRPC are developed and conducted, we envision that it will not take long before these questions are answered and new ones arise.

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Enzalutamide in Metastatic Castration Resistant Prostate Cancer

10

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Introduction: Metastatic Castration Resistant Prostate Cancer: Continuous Dependence on Androgen Receptor Signaling

Prostate cancer (PCa) is the most prevalent non-skin cancer in males, and behind lung cancer, is the second leading cause of cancer death among men in North America [1, 2]. In 1941, Huggins and Hodges successfully treated patients with metastatic PCa through surgical castration, providing proof of principle that PCa is critically dependent on androgens [3]. Decades later, androgen deprivation therapy (ADT) either via gonadotropin releasing hormone (GnRH) manipulation or bilateral orchiectomy remains the standard of care for initial treatment of PCa [4]. However, selective pressure of androgen deprivation eventually leads to a hormone resistant state, a lethal disease phenotype termed metastatic castration resistant prostate cancer (mCRPC), with an expected survival between 2–3 years [5].

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Historically, it was understood that once PCa progressed on ADT, other modalities of androgen receptor (AR) signaling inhibition provided only marginal benefit. Androgen receptor antagonists such as bicalutamide and the lyase inhibitor, ketoconazole, have been successful in generating prostate-specific antigen (PSA) responses in men with CRPC, but have never demonstrated a survival benefit [6, 7]. Many mutations involving the androgen receptor (AR) have since been implicated in the progression and survival of mCRPC, providing molecular insight into identifying actionable targets for development of novel therapies [8]. Some of the proposed mechanisms for mCRPC point to aberrant AR activity, including AR overexpression, internal androgen synthesis, dysregulation of the balance between AR coactivators and corepressors, and constitutively active splice variants [9–14].

Advances in the understanding of castration resistance have led to a renaissance in the development novel androgen receptor signaling inhibitors in clinical testing. The efficacy of these new therapies confirms that a subset of mCRPC continues to be critically dependent on the androgen signaling pathway. It was in this context that enzalutamide, formerly known as MDV3100, was developed.

Development of Enzalutamide

Preclinical Data

Enzalutamide, an androgen receptor signaling inhibitor, was originally selected for clinical development because of its lack of AR agonist effects. Compared to bicalutamide, enzalutamide binds to the AR with a significantly higher affinity [15]. Additionally, in preclinical models, it exhibited potent inhibition of the nuclear translocation of AR, DNA binding, co-activator recruitment activities of AR, and even retains significant preclinical activity even in the presence of AR gene amplification. Most importantly, following exposure to enzalutamide, tumor responses were observed in castration resistant disease models.

Early Phase Clinical Studies

Promising preclinical results led to an initial phase I/II study, with 140 patients with CRPC (78 % with metastatic disease) received daily doses ranging from 30 to 600 mg [16]. The primary objectives of this study were to evaluate its safety and tolerability, as well as to determine its maximally tolerated dose. In the phase I portion, all dose levels induced antitumor effects, and importantly demonstrated a favorable safety profile. The maximum determined dose was 240 mg daily, above which no additional antitumor effect was observed, and led to increasing drug discontinuations due to adverse effects.

In the phase II portion of the trial, the median time to PSA progression, as determined by a 25 % increase in PSA from post-treatment nadir, was 32 weeks

(95 % CI: 21–45), with no statistically significant difference based on prior exposure to chemotherapy. Median time to radiographic progression was not reached in patients without prior chemotherapy exposure, and was 29 weeks (95 % CI: 24–59) in those with prior chemotherapy exposure ($p = 0.01$). Over half of the patients enrolled in the study achieved a PSA decline of greater than 50 %. While there was no difference in maximum PSA response in patients with and without prior chemotherapy, a higher percentage of chemotherapy-naïve patients had maintained at least a 50 % decrease in PSA at twelve weeks compared with those who had prior chemotherapy. Furthermore, a subset of patients had definitive radiographic responses in measurable soft tissue disease, seen in both chemotherapy naïve and treated disease. The disparity between patients with and without prior chemotherapy suggests some measure of cross-resistance between enzalutamide and chemotherapy, although responses were seen in both groups.

The most common grade 3 or 4 adverse event observed was fatigue (11 %); this was observed in a dose-dependent manner, and occurred after four weeks when plasma concentration of the drug had reached steady state. Additionally, there were two witnessed seizures and one possible seizure observed, at doses higher than 360 mg daily, and was regarded with concern. Following these events, all patients in the trial were instructed to lower their dosage to 240 mg daily. Otherwise, enzalutamide therapy was well tolerated.

Phase III and Randomized Data

The AFFIRM Study

The promising clinical activity exhibited in the early phase study led to the development of two randomized studies, AFFIRM and PREVAIL. AFFIRM was a randomized, double-blind, placebo-controlled phase III trial comparing the efficacy of enzalutamide with placebo in mCRPC patients who had received docetaxel previously [17]. With the primary endpoint of overall survival (OS), 1199 patients were randomized in a 2:1 ratio to receive either enzalutamide at 160 mg daily ($N = 800$) or placebo ($N = 399$).

Interim analysis was planned after 520 deaths (planned analysis = 650). Median OS was 18.4 months in the enzalutamide group, compared with 13.6 months in the placebo group (HR = 0.63; 95 % CI: 0.53–0.70; $p < 0.001$). At the time of analysis, of the 800 patients in the intention-to-treat group, 308 had died, and of the 399 patients in the placebo group, 212 had died (38 % vs. 53 %). A statistically significant OS benefit was observed across all subgroups of patients, and maintained even after adjustment for stratification factors and baseline prognostic factors in a multivariate survival analysis (HR = 0.58; 95 % CI: 0.49–0.70; $p < 0.001$).

Enzalutamide demonstrated superior efficacy across all secondary endpoints compared to the placebo (Table 10.1): median PFS (8.3 vs. 2.9 months, HR = 0.40; 95 % CI: 0.35–0.47; $p < 0.001$), median time to PSA progression was 8.3 versus

Table 10.1 Secondary endpoints, AFFIRM and PREVAIL [17, 19]

End point	AFFIRM (enzalutamide vs. placebo)	PREVAIL (enzalutamide vs. placebo)
Radiographic PFS* (m)	8.3 versus 2.9 (HR = 0.40; 95 % CI: 0.35–0.47; p < 0.001)	NR versus 3.9 (HR = 0.19; 95 % CI: 0.15–0.23; p < 0.001)
Time to PSA progression (m)	8.3 versus 3.0 (HR = 0.25; 95 % CI: 0.20–0.30; p < 0.001)	11.2 versus 2.8 (HR = 0.17; 95 % CI: 0.15–0.20; p < 0.001)
Time to first SRE (m)	16.7 versus 13.3 (HR = 0.69; 95 % CI: 0.57–0.84; p < 0.001)	31.1 versus 31.3 (HR = 0.72; 95 % CI: 0.61–0.84; p < 0.001)
≥50 % decline in PSA (%)	54 versus 2; p < 0.001	78 versus 3; p < 0.001
≥90 % decline in PSA (%)	47 versus 1; p < 0.001	47 versus 1; p < 0.001
Objective soft tissue response (%)	29 versus 4; p < 0.001	59 versus 5; p < 0.001
Patients with improved quality of life (%)	43 versus 18; p < 0.001	N/A
Most common AE in enzalutamide group (%)	Fatigue [37], diarrhea [24], hot flash [23]	Fatigue [39], back pain [30], constipation [25]
Number of seizures in enzalutamide group	5	1

* Radiographic PFS was a coprimary endpoint in PREVAIL
M months, *NR* Not reached, *PSA* Prostate Specific Antigen, *SRE* Skeletal related events

3.0 months, HR = 0.25; 95 % CI: 0.20–0.30; p < 0.001), median time to SRE (16.7 vs. 13.3 months, HR = 0.69; 95 % CI: 0.57–0.84; p < 0.001). Enzalutamide was also associated with superior PSA response, with over half of patients with >50 % decline in PSA from baseline, and one-quarter of patients achieved >90 % decline in PSA from baseline (placebo: 2 and 1 % of patients, p < 0.001 for both measurements). Complete or partial soft tissue response was observed in 29 % of patients with soft tissue disease in the enzalutamide group compared with 4 % in the placebo group (p < 0.001). Improvement in quality of life was observed in 43 % of eligible patients in the enzalutamide group compared with 18 % in the placebo group (p < 0.001).

In addition to demonstrating significant clinical efficacy, AFFIRM confirmed the favorable safety profile seen in the earlier phase study. Adverse events occurring more frequently in the enzalutamide group included fatigue, diarrhea, hot flash, musculoskeletal pain and headaches. The incidence of discontinuation due to adverse events or death was comparable to placebo (8 % vs. 10 %). Of note, five patients (0.6 %) in the enzalutamide group experienced seizures, although several of the patients had potentially predisposing risk factors, including brain metastasis. One hypothesized mechanism for the increased number of seizures is the inhibition of gamma-aminobutyric acid channels, though this has not been validated. Seizures, though rare, can be a potentially dangerous toxicity and requires close monitoring. However, the treatment is generally well tolerated. These results led to its regulatory approval by the Federal Drug Administration for treatment of patients with mCRPC after progression on docetaxel therapy [18].

The PREVAIL Study

Since its regulatory approval for patients with post-docetaxel mCRPC, its favorable toxicity profile has enticed earlier enzalutamide use in chemotherapy-naïve disease with evidence of definitive benefit. PREVAIL is a randomized, placebo-controlled phase III trial evaluating the efficacy of enzalutamide in chemotherapy-naïve patients with mCRPC [19]. With co-primary endpoints of radiographic PFS and OS, 1717 patients were randomized to receive either enzalutamide or placebo at a daily dose of 160 mg (N = 872) or placebo (N = 845).

At interim analysis (516 of 540 planned death and 410 of 249 radiographic PFS had occurred), the median radiographic PFS was not yet reached in the enzalutamide group, compared with a median PFS of 3.9 months in the placebo group (HR = 0.19; 95 % CI: 0.15–0.23; $p < 0.001$). The median OS in the enzalutamide group was 32.4 months compared with 30.2 months, with a 29 % reduction in risk of death (HR = 0.71; 95 % CI: 0.60–0.84; $p < 0.001$). The beneficial effect of enzalutamide on both co-primary endpoints remained favorable across all patient subgroups. Enzalutamide therapy was also associated with a favorable outcome compared to placebo in all secondary planned endpoints.

In the PREVAIL study, enzalutamide demonstrated a very similar toxicity profile to that seen in AFFIRM. Overall, 43 % of patients in the enzalutamide group experienced an adverse event of grade 3 or higher, compared with 37 % in the placebo group, though the authors noted that the patients in the enzalutamide group had approximately one extra year of the safety-reporting period. This hypothesis was valid, as patients in the enzalutamide group had a median time to grade 3 or higher adverse event of 22.3 months compared with 13.3 months in the placebo group. Adverse events occurring more frequently in the Enzalutamide group (after adjusting for increased length of exposure to enzalutamide) were hot flashes, hypertension and falls. Notably, there was only one seizure in the enzalutamide group compared with one seizure in the placebo group, and both patients had a prior history of seizure unknown to investigators.

Based on results from PREVAIL, enzalutamide received FDA approval for treatment in patients with docetaxel naïve mCRPC. Importantly, with its favorable toxicity profile and the co-administration of glucocorticoid not required (e.g. docetaxel, abiraterone), it has become an increasingly attractive treatment option for patients with chemotherapy naïve mCRPC.

While enzalutamide demonstrated its efficacy over the use of placebos, it had never been tested against other anti-androgens, though they are commonly used in metastatic CRPC. However, the American Urological Association only recommends its use in this setting with a Grade C level of evidence, all of it from single-arm, non-randomized trials, with a PSA reduction benefit only seen in 20–40 % of patients for a limited duration [20].

The TERRAIN trial was a randomized, double-blind phase II trial comparing combined androgen blockade with enzalutamide 160 mg daily versus bicalutamide 50 mg daily in patients with metastatic CRPC [21]. With a primary endpoint of PFS, a total of 375 patients were randomized, with 184 allocated to the

enzalutamide study group and 191 allocated to receive bicalutamide. Secondary endpoints included investigator-reviewed PFS, time to PSA progression, PSA response by week 13 and best PSA response.

With a median follow-up of 20 months in the enzalutamide group and 16.7 months in the bicalutamide group, enzalutamide was associated with a much improved PFS, with a median PFS of 15.7 months compared with 5.8 months for bicalutamide (HR = 0.44; 95 % CI: 0.34–0.57; $p < 0.0001$). Enzalutamide's benefit was also seen in all secondary endpoints. Enzalutamide continued to demonstrate a favorable safety profile. Adverse events occurring more frequently in the enzalutamide group included fatigue, hypertension, hot flushes, diarrhea, weight decrease and pain in the extremities and back. The only adverse events of grade 3 or higher that occurred more frequently in the enzalutamide group were hypertension and back pain.

STRIVE was another randomized phase II study that demonstrated enzalutamide's superiority over bicalutamide in patients with CRPC, using the same primary endpoint of PFS, though it included patients with non-metastatic disease [22]. In the subgroup of patients with metastases (257 out of 396), enzalutamide demonstrated similar effects to the TERRAIN population, with a median PFS of 16.5 months compared with 5.5 months for bicalutamide (HR = 0.24; 95% CI: 0.17–0.34; $p < 0.05$).

The results of TERRAIN and the metastatic subgroup in STRIVE further strengthen the indication to use enzalutamide in patients with metastatic CRPC, and finally provide randomized, definitive evidence that bicalutamide should not be used in this setting.

Overcoming Enzalutamide Resistance

Several mechanisms of enzalutamide resistance have been identified, mainly by subverting the interaction between enzalutamide and the AR (Fig. 10.1). Among them are glucocorticoid receptor (GR) escape, mutations that confer agonistic properties to enzalutamide, and constitutively active AR splice variants, which lack a ligand-binding domain (LBD) [23–25].

GR Upregulation

Arora et al. reported that elevated expression of GR was associated with clinical resistance to enzalutamide [23]. Patients who had tumors with elevated levels of GR (≥20 % of cells at baseline) were all poor responders to treatment of enzalutamide, and at 8 weeks following the initiation of enzalutamide, poor responders had high levels of GR positive cells compared with good responders (29 % vs. 8 %; $p = 0.009$). The GR was able to activate certain genes that are normally driven by the AR due to overlapping target specificity. Furthermore, in preclinical models, the

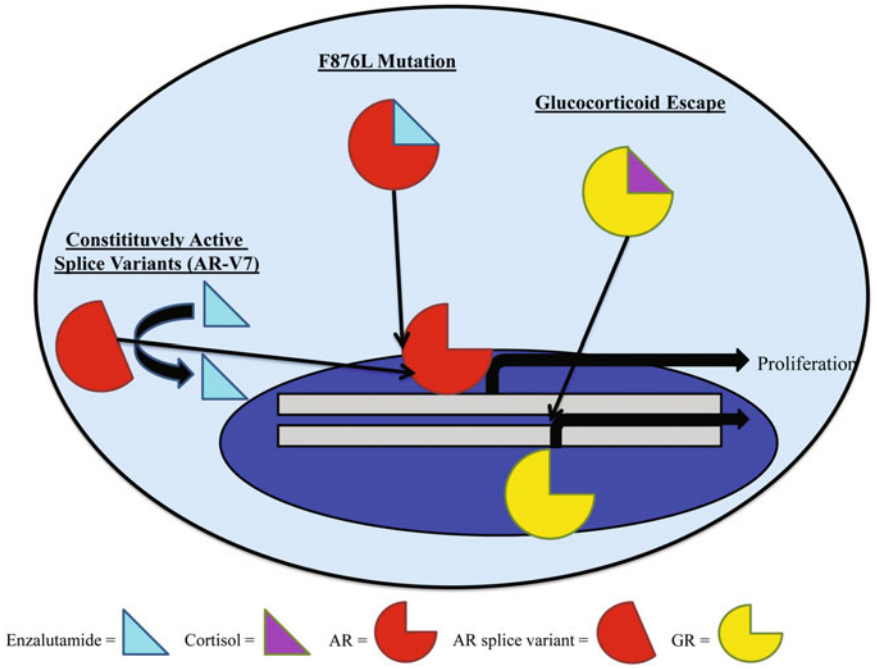

Fig. 10.1 Mechanisms of enzalutamide resistance in prostate cancer. Mechanisms of enzalutamide resistance: constitutively active AR splice variants, F876L point mutation that confers partial agonism to enzalutamide, and glucocorticoid escape

GR agonist dexamethasone was sufficient to confer enzalutamide resistance, whereas a GR antagonist restored sensitivity. The authors demonstrated that the mechanism of glucocorticoid escape was dependent on upregulated GR expression alone, and did not reflect AR function restoration, as many downstream effects of the AR were still inhibited in the anti-androgen resistant tumors.

AR Mutation

Various AR mutations have been identified in enzalutamide resistance disease. One example is a missense mutation in the AR F876L, which confers agonistic properties to enzalutamide, much like the mutations that confer agonistic properties to bicalutamide [25]. Joseph et al. created enzalutamide-resistant cell lines through prolonged in vitro exposure. In three of the resistant cell lines, enzalutamide displayed partial agonist activity, and through sequencing of the AR gene, it was determined that all three of these cell lines contained the F876L mutation. Further cell lines were designed to contain an overexpression of the F876L mutation, in

which enzalutamide induced strong transcriptional and proliferative activity. F876L mutations also conferred resistance in vivo, as enzalutamide failed to demonstrate antitumor activity in mice injected with AR F876L cells.

AR Splice Variants

Androgen receptor splice variant-7 (AR-V7) is a truncated form of the androgen receptor that lacks the ligand-binding domain, the target of enzalutamide, but remains constitutively active as a transcription factor. Antonarakis et al. demonstrated that patients who harbored detectable levels of splice variant AR-V7 in circulating tumor cells (CTC), had a markedly diminished response to enzalutamide compared to those without it [26]: None of the 12 patients with detectable AR-V7 achieved a PSA response, compared to 53 % response rate in those negative for AR-V7 ($p = 0.004$). Median PSA-PFS was 1.4 months in those with positive AR-V7 compared to 6.0 months with negative AR-V7 (HR 7.4, 95 % CI: 2.7–20.6; $p < 0.001$), and remained statistical significant after adjusting for full-length AR mRNA and prior abiraterone use (HR = 3.0; 95 % CI: 1.0–9.2; $p = 0.046$). Median clinical or radiographic PFS was 2.1 months in AR-V7 positive patients compared with 6.1 months in AR-V7 negative patients (HR = 8.5; 95 % CI: 2.8–25.5; $p < 0.001$). With a median follow-up of 8.4 months, median OS was 5.5 months in AR-V7 positive patients and was not reached in AR-V7 negative patients (HR = 6.9; 95 % CI: 1.7–28.1; $p = 0.002$).

Enzalutamide in Practice Today

Recent regulatory approval of therapies with distinct mechanisms of action has raised questions about the optimal sequence in the treatment of prostate cancer. Two large randomized phase III studies have now established docetaxel chemotherapy as the standard of care in first line treatment of hormone sensitive metastatic disease with high tumor burden, but for those with slow growing or has low burden of disease, choice of therapy is less clear [27, 28]. Optimal use of enzalutamide in the landscape of prostate cancer treatment, whether before, after, or even concurrently with other therapies, is subject to further investigation.

Pre-chemotherapy versus Post-chemotherapy

Currently the NCCN guidelines recommend both enzalutamide or abiraterone acetate as first line treatment for mCRPC, and for the use of more toxic chemotherapy for those resistant to these agents. This is reasonable, but lack of precise biomarkers to predict treatment response leaves the choice of treatment “personalized” to the clinician based on individual preference.

Before, After or Instead of Abiraterone Acetate?

There have been no prospective randomized trials completed at this time to address the sequence of enzalutamide and abiraterone acetate treatment. However, several retrospective studies in both chemotherapy–naïve and refractory disease have provided hypothesis-generating results that will require confirmation in large randomized studies. To date, there have been 5 published studies examining the efficacy of enzalutamide after disease progression with abiraterone acetate in patients with docetaxel refractory disease. Cumulatively, patient treated in this setting had a PSA decline >50 % between 10–29 % [29–33]. The numbers were strikingly similar in chemotherapy-naïve patients, with 25–34 % of patients having a PSA decline >50 % [34, 35].

Studies examining treatment of abiraterone acetate in enzalutamide refractory disease were even more disappointing, raising the question whether cross-resistance pattern may differ when different sequences of the two androgen signaling inhibitors are used. Two retrospective studies examined the efficacy of abiraterone acetate therapy in patients with docetaxel and Enzalutamide refractory disease [36, 37]. Noonan et al. reported that the median PFS was a modest 3.6 months in this setting. Another study reported by Loriot et showed a PFS of 2.7 months, and additionally only 8 % of the patients had >50 % decline in PSA after 4 weeks of treatment. To date, there has been no report which has examined the role of abiraterone acetate after enzalutamide in chemotherapy-naïve patients. Importantly, docetaxel therapy after progression on either abiraterone acetate or enzalutamide appears to be more promising, with 55 % of patients having a PSA decline >50 % with docetaxel therapy following therapy with either therapy [38]. The optimal sequence of enzalutmide use will have to be determined in future studies.

Future Directions

Results from CHARRTED and STAMPEDE, which showed patients who received docetaxel in metastatic hormone sensitive prostate cancer had a survival benefit >1 year compared to those who may have received the therapy later, highlights the importance of treatment sequence in treating patients with metastatic prostate cancer. Effort has begun to understand how and when to best use Enzalutamide. Important questions include but not limited to: benefit in earlier disease stage, concurrent use in combination with other approved therapies, sequence in treatment of mCRPC, and value of re-treatment after initial progression (see Table 10.2). It is also important is to continue identifying enzalutamide resistance mechanisms, leading to development of corresponding targeting therapy. One opportunity is to target AR splice variant; while AR-V7 splice variant can evade drug binding at the C-terminus, one novel approach is to target DNA-binding at the N-terminal domain of the AR [39]. In fact, one such therapy is EPI-506, and is currently in early clinical trial testing [40]. The results of these important clinical studies will help shape our treatment approach going forward.

Table 10.2 Actively phase III/IV studies evaluating enzalutamide treatment in mCRPC (https://clinicaltrials.gov)

NCT#	Study	Interventions	Disease state
NCT02003924	Safety and efficacy of enzalutamide in patients with nonmetastatic CRPC	Enzalutamide vs. placebo	Nonmetastatic CRPC
NCT02288247	A study to assess the benefit of treatment beyond progression with enzalutamide in men who are starting treatment with docetaxel after worsening of their prostate cancer when taking Enzalutamide alone	Enzalutamide plus docetaxel and prednisolone vs. placebo plus docetaxel and prednisolone	Enzalutamide resistant, chemotherapy-naïve mCRPC
NCT01949337	Enzalutamide with or without abiraterone and prednisone in treating patients with metastatic CRPC	Enzalutamide plus abiraterone plus prednisone vs. enzalutamide plus placebo plus prednisone	Chemotherapy-naïve mCRPC
NCT02294461	An Asian study to evaluate efficacy and safety of oral enzalutamide in progressive metastatic	Enzalutamide versus placebo	Chemotherapy-naïve, mCRPC
NCT01977651	A study to evaluate the potential increased risk of seizures among metastatic CRPC patients treated with enzalutamide	Enzalutamide	mCRPC
NCT02441517	Enzalutamide Re-treatment in Metastatic Castration-resistant Prostate Cancer After Docetaxel Treatment in Patients Who Have Previously Received Enzalutamide	Enzalutamide	mCRPC, docetaxel refractory

Another important direction is to accurately assess disease heterogeneity prior to selecting the next line of therapy. With substantial advances in the molecular understanding of prostate cancer, this has led to an unmet need to develop real time predictive markers which can guide practitioners to make the best therapeutic choice. Although serial metastatic biopsies are ideal, given its invasive nature, this is not feasible in practice. One promising approach is via "liquid biopsy," with collection of circulating tumor cells via peripheral blood sample, molecular profiling of CTCs provides a glimpse of hope that this may become a reality in the near future [41].

Conclusion

Enzalutamide has demonstrated favorable efficacy and tolerability in the treatment of patients with mCRPC, both for patient who were naïve or refractory to docetaxel chemotherapy. However, to optimize its effectiveness in the treatment of prostate cancer, more studies are needed in order to determine its best use. Continued investigation of enzalutamide resistance pathways and identifying actionable targets will be key in development of novel treatments in this lethal disease.

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Radium-223 in Metastatic Castrate Resistant Prostate Cancer

11

Tu Dan, Noelle Williams and Robert B. Den

Introduction

Metastatic disease continues to be a leading cause of morbidity and mortality in men with prostate cancer. In 2015, it is estimated that approximately 30,000 men will succumb to their disease due to metastatic spread of cancer [1]. The current standard of care for newly metastatic patients includes surgical or medical castration with luteinizing hormone releasing hormone (LHRH) analogs or antagonists. However, following initial anti-androgen therapy, patients often progress to castrate resistant disease. Currently, consensus guidelines regarding treatment after castrate resistance vary widely and remain an area of evolving investigation [2].

Over the last decade, the treatment landscape for patients with metastatic castrate resistant disease has drastically changed, with several novel agents demonstrating an improvement in overall survival in large, multi-institutional randomized trials. These new agents include the incorporation of newer cytotoxics [3], next generation anti-androgens [4, 5], immunotherapeutics [6], and radiopharmaceuticals [7]. Of these available treatments, the first in class radiopharmaceutical radium-223 has emerged as the only bone-directed treatment option demonstrating an improvement

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in overall survival. In this chapter we will discuss the development of radium-223 in prostate cancer, its unique mechanism of action, clinical outcome data, practical use, and future directions.

Bone Metastases in Prostate Cancer

Unlike other neoplasms of the male genitourinary system, metastatic prostate cancer overwhelmingly involves osseous structures, particularly the axial skeleton including the bony pelvis, ribs, and vertebral bodies of the spinal column. Metastatic lesions in these locations are a leading cause of morbidity and mortality in patients with prostate cancer due to the significant impact on functionality and quality of life. The exact mechanism in which prostate cancer preferentially involves osseous structures has yet to be elucidated, however it likely involves the complex interplay between circulating tumor cells and bone microenvironment [8]. Bone homeostasis is a complex cellular process regulated by both osteoclast and osteoblast activity. With increased osteoblast activity, calcium utilization is increased making calcium-mimetics an effective targeted treatment strategy in malignancies with a significant osseous blastic component. The vast majority of men with prostate cancer present with clinically localized disease. Given that the majority of those with metastatic disease will eventually develop some evidence of bone metastases (subclinical or symptomatic) suggests that bony dissemination may occur early in the disease process [9]. It is classically thought that metastatic prostate cancer yields purely osteoblastic lesions, however, emerging evidence suggests that there are dual components of both lytic and blastic bone formation [10].

Osseous metastases contribute to local and systemic symptomatology, contribute to a patient's burden of disease, and impact quality of life. Osseous metastases can lead to paraneoplastic syndromes including hypercalcemia secondary to deranged humoral processes involved in bone remodeling. Diffuse osseous involvement can also lead to marrow suppression and pancytopenia. In patients treated with chemotherapy, this effect becomes deleteriously cumulative, leading to prolonged cytopenias sometimes requiring transfusion dependence. Bone metastases can be extremely painful, requiring high-dose narcotics for pain management. In addition, involvement of the vertebral column can lead to spinal cord compression causing paresis and paralysis. Loss of mobility also occurs with involvement of weight-bearing structures leading to pathologic fractures. These events collectively are often referred to as skeletal-related events (SREs) or symptomatic skeletal events (SSEs), and their reduction is used as an endpoint a number of clinical trials.

In the ALSYMPCA trial, radium-223 was compared to placebo in men with castrate resistant metastatic prostate cancer. SSEs were defined as the use of external beam radiation therapy to relieve bone pain, occurrence of new symptomatic pathological bone fractures (vertebral or non-vertebral), occurrence of spinal cord compression, or tumor-related orthopedic surgical intervention [11]. Further, the extent of osseous involvement can be an independent predictor of

overall survival [12]. Given the systemic and complex nature of managing painful bone metastases, targeted therapeutics have emerged as an attractive treatment option to improve quality of life and overall survival in this disease.

Radiopharmaceuticals in Prostate Cancer

Nucleotide scans targeting the bone remodeling system have long been used in the evaluation of metastatic prostate cancer. The most common of these utilize technetium-99 methylene diphosphonate. Newer modalities such as sodium fluoride PET and 18-fluorodeoxyglucose PET have also demonstrated promise due to their detection sensitivity [13]. Given that radionuclides have demonstrated sensitivity and specificity for detecting osseous disease, a natural extension of their application has been for therapeutic purposes.

The use of therapeutic radiopharmaceuticals in prostate cancer has a long history and a number of randomized controlled trials have demonstrated their efficacy across several endpoints. Their application thus far has mostly been studied in the palliative setting. Currently used therapeutic radiopharmaceuticals in the treatment of metastatic prostate cancer include strontium-89, samarium-153, rhenium-186 and rhenium-188, and most recently, radium-223. The physical characteristics of these agents are shown in Table 11.1.

The first of these agents, strontium-89, received FDA approval in 1993 for use in the treatment of painful bone metastases [14]. Strontium-89 is a calcium-mimetic that decays as a pure β emitter and is incorporated into bone following intravenous administration. When compared to normal bone, strontium has a 10-fold increase in uptake into bone containing metastatic disease [15]. There have been a number of randomized trials evaluating the efficacy of strontium-89 in the palliative setting. In one systematic review, complete pain response was reported from 8 to 77 % with a partial pain response noted in 44 % of patients [16]. The most common toxicities associated with administration include leukopenia and thrombocytopenia.

Samarium-153, a β emitter with 28 % γ emission, was the next radionuclide approved by the FDA. Unlike other therapeutic radionuclides, samarium is not a calcium-mimetic and is complexed with ethylene diamine tetramethylene phosphonate (EDTMP) which rapidly localizes to bone in association with hydroxyapatite. It has a five times greater affinity for tumor as compared to normal bone [17]. Similar to strontium, there are multiple randomized phase III trials demonstrating an improvement in bone pain and reduced analgesic use with the utilization of samarium.

Table 11.1 Physical characteristics of radiopharmaceuticals used in prostate cancer

Radionuclide	Half-life (days)	Decay particle	Tissue penetration (mm)
Radium-223	11.4	Alpha	<0.1
Strontium-89	50.5	Beta	5.5
Samarium-153	1.9	Beta, gamma	2.5
Rhenium-186	3.8	Beta, gamma	4.5
Rhenium-188	0.7	Beta, gamma	11.0

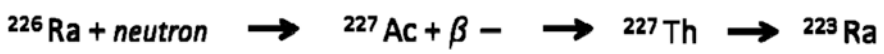

Fig. 11.1 Radium-223 production. Based on data from Ref. [22]

Other radionuclides used include rhenium phydroxyethylidene diphosphonate (HEDP) and isotopes rhenium-186 and rhenium-188. These isotopes are agents that have both significant β and γ emission, allowing for both therapeutic and diagnostic use. When compared for efficacy, all agents demonstrated some evidence for pain relief, but there was no statistical significance between the various agents in terms of pain palliation, analgesic use, or bone marrow toxicity [18].

Unlike the other beta-emitters used in this disease, radium-223 relies on alpha-decay to exert its therapeutic properties. Historically, primary outcomes in studies utilizing beta-emitters have included pain response, decrease in analgesic consumption, and quality-of-life. However, due to its unique properties, clinical efficacy with radium-223 has been measured in terms of overall survival, in addition to improving quality of life.

Radium has 33 known isotopes from ^{202}Ra to ^{234}Ra [19]. The most common and stable form is radium-226 which was initially used as a brachytherapy source in the early 20th century, particularly in the treatment of gynecological cancers [20]. It usefulness as a brachytherapy source relates to its long half-life and relatively high specific activity. Radium-223 is typically formed from radium-226 through a series of reactions involving nuclear bombardment of radium-226 with neutrons to produce radium-227, which in turn eventually decays through beta-emission to radium-223, shown in Fig. 11.1 [21]. The decay of radium-223 results in the emission four alpha particles, rather than beta or gamma emission. The significance of this will be discussed in the next section. As a result, the cytotoxicity from this interaction is over 100-fold more potent than that of beta emission, resulting in a significantly more effective therapy.

Radium-223 Mechanism of Action

As discussed previously, while the majority of radiopharmaceuticals rely on the emission of beta particles for their therapeutic effect, radium-223 utilizes alpha particle emission. Radioactive decay occurs when the nucleus of an unstable isotope loses energy through emission of particles. Radiation may be emitted in the form of alpha (α) particles, beta (β) particles, or gamma (γ) rays. An alpha particle consists of two protons and two neutrons, a β particle is a high energy electron, and a γ ray is described as ionizing electromagnetic radiation. Radioactive decay of radium-223 results in the emission of 4 alpha-particles, which produces approximately 95 % of the energy released by radium-223. Because of their charge and large mass, alpha particles are easily absorbed and can travel only short distances before losing all of their energy [23].

A key feature of alpha particle emission is its high linear energy transfer (LET). Related to LET is the concept of relative biologic effectiveness (RBE). LET refers to the average energy imparted to a medium by a particle per unit track length (classically in units of keV/um). RBE is typically defined as the ratio of a test dose of a radiation required to produce the equal biological effect as a reference dose from standard 250 kVp x-rays. Low LET, or sparsely ionizing radiation, is typically associated with gamma rays and x-rays. High LET radiation is associated with heavy charged ions such as alpha-particles. Other forms of high LET radiation include neutron and carbon ion particles. High LET particles interact with the nucleus of matter and produce complex, clustered DNA lesions [24].

Typically high-LET type radiation is associated with a larger RBE. Due to its high LET, the RBE of alpha particles is several fold higher than that of beta emission and gamma rays. An additional advantage of alpha particles is that it has extremely short range of action leading to a dense deposition of energy along a given track. When interacting with DNA from tumor cells, alpha particles produce complex, clustered double-stranded DNA damage that are not repairable, resulting in a high RBE and high cell kill. Due to the range of the interaction, nearby normal tissue such as myeloid cells are preferentially spared, resulting in a high therapeutic ratio. In contrast to alpha particles, β emitters have track lengths that consist of up to a few millimeters, which result in collateral bone marrow toxicity. Furthermore, β particles require increased shielding due to their increased penetration.

The tissue-sparing effect of alpha particles has previously been modeled. In one study, investigators measured distances of hematopoietic stem and progenitor cells from the surface of the bone. The authors found that hematopoietic cells were found to exist along a linear spatial gradient with a significant portion of cells located outside of the range expected for alpha particles [25]. In another study, authors used a Monte Carlo model to determine dose to marrow cells at different distances from the bone surface. Absorbed dose was predominantly deposited near the trabecular surface with the observation that increasing the radioactivity of the administered dose would like not increase marrow toxicity due to the relative sparing of marrow stem cells [26]. Due to these observations, a high therapeutic ratio was expected from the use of alpha particles.

Preclinical Data

Initial experiments leading to the usage of radium-223 in humans were performed in a similar fashion as previous models investigating other calcium mimetics. The goals of the initial studies were to confirm the hypothesis that bone-targeting alpha-emitters could localize to the bone and deliver therapeutically relevant radiation doses. This was first tested in a murine dosimetry model in which tagged radionucleotides At-211 and I-131 bisphosphate were injected and measured in various organs to determine uptake [27]. Both beta-particle- and alpha-particle-emitting compounds demonstrated high in vivo stability and affinity for osseous tissue. These experiments were repeated

in additional murine and canine models using various alpha-emitters as proof of concept that these radionuclides could be preferentially taken up by bone [28, 29]. While a number of alpha emitters have been studied in the past, the production and distribution limitations with short-lived alpha-emitters like At-211, Bi-212, and Bi-213 made them poor candidates for clinical use due to practical and logistical reasons [30]. Radium-223 was eventually chosen due to its favorable decay chain and half-life. Interestingly, due to the poor availability of prostate cancer models of bone metastases, some of the first preclinical efficacy studies of radium-223 were actually performed in nude mice models of breast carcinomas. In these studies, the administration of the higher dose cohort of radium-223 resulting in improved survival of these mice with minimal toxicity [31].

Clinical Data

Based on a strong mechanism of action and favorable preclinical studies, the first phase I trial was conducted in the early 2000s assessing the safety and tolerability of radium-223 (Table 11.2). In a dose-escalation study, 15 prostate and 10 breast cancer patients with evidence of skeletal metastases were enrolled at a single institution and received single injections of radium-223 at dosages of 46, 93, 163, 213, or 250 kBq/kg and followed for 8 weeks [32]. Palliative response was evaluated as a secondary endpoint. In this study, investigators found radium-223 to be extremely well-tolerated with mild and reversible myelosuppression. Importantly, only grade 1 thrombocytopenia was reported. Mild, transient diarrhea was observed in 10 of the 25 patients. In addition, a significant portion of patients reported pain relief with only a single dose of treatment up to 8 weeks following administration.

Given these promising results, a number of phase II trials were initiated. To date, there have been 3 major prospective phase II studies published utilizing radium-223 in the metastatic prostate cancer setting leading to the definitive phase III study. In the first study, in a randomized, double-blind, placebo-controlled, multicenter phase II study, investigators studied the effect of repeated administration of radium-223 doses in men with symptomatic, hormone-refractory prostate cancer [33]. In this study, 64 patients were randomized to placebo versus 4 administrations of

Table 11.2 Key clinical trials utilizing radium-223 in metastatic prostate cancer

Author	Publication date	Phase	Disease site(s)	Primary endpoint
Nilsson et al. [32]	2005	I	Breast, Prostate	Toxicity
Nilsson et al. [33]	2007	II	Prostate	Mean change in bone ALP
Nilsson et al. [34]	2012	II	Prostate	Pain index from baseline
Carrasquillo et al. [48]	2013	I	Prostate	Biodistribution, pharmacokinetics
Parker et al. [35]	2013	II	Prostate	PSA response
Parker et al. [7]	2013	III	Prostate	Overall survival

50 kBq/kg of radium-223. The primary endpoint was mean change in bone alkaline phosphatase (ALP) from baseline to 4 weeks after the last injection. Investigators also looked at time to first SRE, PSA change and overall survival. Results from this trial demonstrated significantly improved ALP levels and time to PSA progression. In addition, investigators found an improvement in overall survival, although the trial was not powered to detect such a change. Observed toxicity was minimal, with no patients discontinuing treatment due to toxicity.

Due to the lack of toxicity seen with the 50 kBq/kg dose, an additional study investigated whether radium-223 could relieve pain in a dose-related manner in patients with metastatic castrate-resistant prostate cancer (mCRPC) and painful bone metastases, whether a pain-relieving effect occurs within each dose-group, and whether pain reduction is associated with improved functional status [34]. A randomized, dose-response, multicenter phase II study of radium-223 for the palliation of painful bone metastases in patients with castrate-resistant prostate cancer was performed. In this study of 100 patients, investigators found that a significant dose response for pain index was seen at week two. Similar to initial phase I data, patients in the high dose cohort (100 kBq/kg) appeared to have the most significant pain relief. This trial also confirmed the safety and tolerability of radium-223 administration, with less than 10 % of patients in all dose groups experiencing a grade 3 hematologic toxicity or higher. Interestingly, unlike pain relief, there did not appear to be a dose response relationship with administered dose and toxicity (no difference in toxicity rates between groups).

The combination of dose escalation with repeated dosing was investigated in another multi-center randomized Phase II study in patients with metastatic prostate cancer [35]. In this double-blind study, 122 patients were randomized to receive 3 injections of radium-223 at 6-week intervals, at doses of 25, 50, and 80 kBq/kg. In this study, PSA decline of 50 % was the primary outcome. Authors reported a significant dose-response in regards to number of patients having 50 % PSA decline, with the greatest decline seen in patients in the 80 kBq/kg group. Similar to previous studies, there was no dose-response relationship between radium-223 dose and adverse hematologic event. It should be noted that none of the phase I or phase II trials have demonstrated a maximum tolerated dose of radium-223, indicating that there may still be room for significant dose-escalation, particularly since several studies have a dose-response relationship with efficacy endpoints and radium-223 dose.

Due to the growing body of phase I and II data, a large international phase III trial was initiated to formally evaluate the efficacy of radium-223. The ALSYMPCA trial randomly assigned 928 men with pain of any intensity related to bone metastases from mCRPC and whose disease had either progressed on docetaxel or who were not docetaxel candidates, to receive 50 kBq/kg of radium 223 intravenously over 1 min each month for 6 doses or placebo IV each month for 6 doses in conjunction with standard care [7]. Men with known visceral metastases were excluded, but malignant lymphadenopathy smaller than 3 cm in short axis diameter was allowed. The primary endpoint was overall survival and the main secondary endpoint was time to symptomatic SRE.

In this landmark trial, the investigators were able to demonstrate a significantly improved overall survival (median, 14.0 months vs. 11.2 months; hazard ratio, 0.70; 95 % confidence interval [CI], 0.55–0.88; two-sided P = 0.002). All main secondary efficacy end points provided support for the benefit of radium-223 plus the best standard of care over placebo plus the best standard of care. Radium-223, as compared with placebo, significantly prolonged the time to the first symptomatic SRE (median, 15.6 months vs. 9.8 months; hazard ratio, 0.66; 95 % CI, 0.52–0.83; P < 0.001), the time to an increase in the total alkaline phosphatase level (hazard ratio, 0.17; 95 % CI, 0.13–0.22; P < 0.001) and the time to an increase in the PSA level (hazard ratio, 0.64; 95 % CI, 0.54–0.77; P < 0.001).

In the updated report on symptomatic skeletal events, SREs occurred in 202 (33 %) of 614 patients in the radium-223 group and 116 (38 %) of 307 patients in the placebo group [11]. Time to first symptomatic SRE was longer with radium-223 than with placebo (median, 15.6 months [95 % CI 13.5–18.0] vs. 9.8 months [7.3–23.7]; hazard ratio [HR] = 0.66, 95 % CI 0.52–0.83; p = 0.00037). The risks of external beam radiation therapy for bone pain (HR 0.67, 95 % CI 0.53–0.85) and spinal cord compression (HR = 0.52, 95 % CI 0.29–0.93) were reduced with radium-233 compared with placebo. Radium-223 treatment did not seem to significantly reduce the risk of symptomatic pathological bone fracture (HR 0.62, 95 % CI 0.35–1.09), or the need for tumor-related orthopedic surgical intervention (HR 0.72, 95 % CI 0.28–1.82).

Assessing Treatment Response

PSA outcomes following radium-223 administration have been variable (Table 11.3). In one of the earlier initial phase II studies investigating single dose escalation, administration of radium-223 had no effect on PSA level [34]. Alternatively, in a phase II study investigating sequential treatment with 4 doses of 50 kBq/kg, investigators found a greater decrease in PSA from baseline (23.8 %, range 98.6–545.6) in the radium-223 group versus an increase of 44.9 % (range 91.3–563.5) in the placebo group (p = 0.003, Wilcoxon ranked-sums test) [33]. Similarly, a phase II study where sequential treatment with dose escalation was used, investigators found a statistically significant dose-response relationship and confirmed 50 % of PSA decline [35]. Median percentage changes were −14.3, −39.6, and −25.3 in the 25, 50, and 80 kBq/kg dose groups, respectively (p = 0.28). In the confirmatory phase III ALSYMPCA trial, a 30 % or greater reduction in PSA blood levels at week 12 was achieved in 16 % of patients in the radium-223 group and in 6 % of patients in the placebo group (P < 0.001). This reduction was sustained 4 weeks after the last injection in 14 % of patients in the radium-223 group and in 4 % of patients in the placebo group (P < 0.001) [7].

In all studies, serum markers of ALP have been shown to be significantly decreased, with most of the phase II studies demonstrating 50 % or greater reduction in ALP in the high dose arms. In the ALSYMPCA trial, a significantly

Table 11.3 PSA control in phase II/III trials utilizing radium-223

Author	Publication date	PSA improvement	PSA endpoints
Nilsson et al. [33]	2007	Yes	Median time to PSA progression remained at 26 weeks for radium-223 versus 8 weeks for placebo (p = 0.040, log rank)
Nilsson et al. [34]	2012	No	PSA levels increased in all dose-groups, from baseline to week 16
Parker et al. [36]	2013	Yes	Proportion of patients with a confirmed reduction of ≥50 % PSA significantly increased with increasing doses
Parker et al. [7]	2013	Yes	Time to PSA progression (hazard ratio, 0.64; 95 % CI, 0.54–0.77; P < 0.001)

higher proportion of patients in the radium-223 group than in the placebo group had a response according to the total ALP level (≥30 % reduction, P < 0.001) and normalization of this level (P < 0.001). A 30 % or greater reduction in PSA blood levels at week 12 was achieved in 16 % of patients in the radium-223 group and in 6 % of patients in the placebo group (P < 0.001). This reduction was sustained 4 weeks after the last injection in 14 % of patients in the radium-223 group and in 4 % of patients in the placebo group (P < 0.001).

Despite its mechanism of action, response to treatment by evaluation with technetium-99 methylene diphosphonate bone scintigraphy scans has not been well described. In the ALSYMPCA trial and others, bone response has been primarily measured by serum markers. In one small single-institution series, investigators found that 10 of 12 evaluable patients demonstrated decreased radiotracer uptake in existing lesions one month following the last dose of treatment. However, new areas of uptake were also noted to develop in 11 of 12 of these patients [36]. Current studies are investigating the use of newer modalities such as (18)F-choline PET/CT in treatment response [37].

Toxicity

In the ALSYMPCA trial, radium-223 was associated with an overall low incidence of grade 3 or 4 myelosuppression (thrombocytopenia, 6 % vs. 2 % placebo; neutropenia, 2 % vs. 1 %; and anemia, 13 % vs. 13 %) and a low incidence of grade 3 or 4 gastrointestinal AEs (diarrhea, 2 % vs. 2 % placebo; vomiting, 2 % vs. 2 %; and constipation, 1 % vs. 1 %). In the 3 year follow up for adverse events, 27 (7 %) of 405 patients receiving radium-223 and 8 (5 %) of 167 patients receiving placebo had 42 treatment-related adverse events [38]. Myelosuppression incidence was ≤3 %. No patients developed AML, MDS, or primary bone cancer.

In the pre-specified subgroup analysis of patients with or without previous docetaxel use, investigators found that patients who previously received docetaxel treatment had an increased risk of hematological toxic effects of any grade compared to those with no previous docetaxel use [39]. However, frequency of grade

3–4 thrombocytopenia appeared to only be increased in the subgroup of patients receiving previous docetaxel. Frequencies of grade 3–4 neutropenia and anemia in the docetaxel subgroups were similar, although more patients who had received previous docetaxel required a blood transfusion. The investigators did not report any differences in non-hematological adverse events between the subgroups.

Patient Selection and Management

Typical laboratory work-up prior to initiation of treatment includes baseline hematologic evaluation. The absolute neutrophil count (ANC) should be $\geq 1.5 \times 10^9$/L, platelet count $\geq 100 \times 10^9$/L, and hemoglobin $\geq 50 \times 10^9$/L. Following initiation of treatment, before each monthly treatment, ANC should be $\geq 1.0 \times 10^9$/L, and platelet count $\geq 50 \times 10^9$/L. If count recovery does not occur within 6 to 8 weeks after administration, it is recommended that treatment be discontinued. However, it should be noted in that in the initial dose-escalation studies leading to its approval, there was no true dose-limiting toxicity seen, even with the highest dose cohorts.

Radium-223 is excreted via the intestinal system, which can manifest as diarrhea, nausea or vomiting. Careful monitoring of the patient's oral intake and fluid status is important to prevent dehydration. There are no contact restrictions or isolation precautions for patients receiving radium-223. Patients are instructed to follow good hygiene during the 6 months of therapy and 1 week after completion of treatment to minimize radiation exposure to household members and caregivers. In regards to shielding requirements, alpha particles travel only short distances in air and are easily stopped by a thin sheet of paper.

Sequencing

Since 2004, six new systemic therapies have been FDA approved for patients with mCRPC. Each agent was developed independently, with no formal evaluation regarding sequencing and integration with other established agents such as docetaxel. Currently, there are limited consensus recommendations regarding sequencing of these newly approved therapeutic agents due to their novel availability [40, 41].

In the pre-specified subgroup analysis from the ALSYMPCA trial, investigators reported that radium-223 prolonged median overall survival irrespective of previous docetaxel use [39]. In comparison, in analogous studies leading to the approval of abiraterone acetate and enzalutamide, a benefit was similarly seen in both docetaxel-naive and post-docetaxel settings. Interestingly, when comparing HRs for death studies utilizing these next-generation anti-androgens to that of radium-223, active treatment versus placebo appears to be similar in both groups (0.63–0.75) [4, 5, 42, 43]. Thus, it appears that even when comparing systemic therapies with distinct mechanisms of action, clinical benefit is essentially independent from

previous docetaxel exposure. However, despite demonstrating a survival advantage, it was noted that the benefit in delaying SREs was only significant in the patients without prior exposure to docetaxel.

Combination Treatment

Given the lack of data regarding sequencing of treatment, it should be no surprise that there are fewer data regarding the use of combination treatment. Due to promising results from each of the newly approved individual agents, it has been suggested that the rational use of combination treatment may lead to even greater response rates and clinical outcomes with minimal increase in toxicity. Unlike traditional cytotoxic systemic treatments, several of these new agents demonstrate non-overlapping mechanisms of action with distinct toxicity profiles (Table 11.4).

The use of cytotoxic therapy and radioisotope treatment has previously been investigated with early generation radiopharmaceuticals. In a randomized phase II study, bone-targeted therapy for advanced prostate cancer using Sr-89 plus doxorubicin weekly was associated with improved survival versus doxorubicin alone [44]. Authors from this study suggested that the combination of systemic therapy with bone targeted treatment improved outcomes due to dual-targeting of both epithelial and stromal components of the disease. By targeting the primary tumor as well as the metastatic niche, a synergistic treatment response was obtained. An approach combining cytotoxics with radium-223 could be even more promising due to lesser toxicity with alpha-emitters.

Next-generation anti-androgens such as abiraterone and enzalutamide also appear to be attractive candidates for combination therapy with radium-223. In previous studies utilizing abiraterone and enzalutamide in the pre-chemotherapy setting, no significant hematological toxicity was reported [4, 5]. As there appear to be non-overlapping toxicities, a novel treatment strategy with concurrent combination therapy may be a reasonable option. Recently, in a single-institution retrospective study, concurrent administration of radium-223 and next generation anti-androgen therapies appears to be well tolerated with similar toxicities to standard administration of radium-223 alone [45]. This particular cohort of patients represents a high-risk,

Table 11.4 Systemic therapies with proven survival advantage in mCRPC

	Experimental group	Control group	Primary endpoint
TAX-327	Docetaxel	Mitoxantrone	Overall survival
TROPIC	Cabazitaxel	Mitoxantrone	Overall survival
COU-AA-301	Abiraterone	Placebo	Overall survival
COU-AA-302	Abiraterone	Placebo	Overall survival
AFFIRM	Enzalutamide	Placebo	Overall survival
PREVAIL	Enzalutimide	Placebo	Overall survival
ALSYMPCA	Radium-223	Placebo	Overall survival

heavily pretreated group of patients with advanced metastatic disease and significant marrow burden. Despite these risk factors, hematologic toxicity was modest and was in the range expected for this risk group based on previous trials.

Ongoing Trials

Next-generation anti-androgen therapies with abiraterone and enzalutamide represent unique opportunity for combination therapy as the side effect profile from these therapies tends to be mild for most patients. At the time of this publication, there are a number of clinical trials underway investigating the use of concurrent anti-androgen and radiopharmaceutical treatment. In one of the largest of these trials, patients will be randomized between radium-223 alone, radium-223 with abiraterone, or radium-223 with enzalutamide (NCT02034552) (Table 11.5).

Table 11.5 Current trials investigating combination therapy with radium-223

Agents tested	Title	Primary endpoint	Open date
Radium-223 Tasquinod	A study of Radium-223 in combination with Tasquinimod in bone-only metastatic castration-resistant prostate cancer	Safety	March 2015
Radium-223; Sipuleucel-T	Ph 2 study of sipuleucel-T W/or W/O Radium-223 in men with asymptomatic or minimally symptomatic bone-MCRPC	Immune response	July 2015
Radium-223; Pazopanib; Sorafenib	Exploratory study of radium-223 and vascular endothelial growth factor-targeted therapy in patients with metastatic renal cell carcinoma and bone metastases	Biomarkers	April 2015
Radium-223; Abiraterone	Radium-223 Dichloride and Abiraterone Acetate compared to placebo and Abiraterone Acetate for men with cancer of the prostate when medical or surgical castration does not work and when the cancer has spread to the bone, has not been treated with chemotherapy and is causing no or only mild symptoms	Symptomatic skeletal event	March 2014
Radium-223; Enzalutimide	A randomized phase IIa efficacy and safety study of Radium-223 Dichloride with Abiraterone Acetate or Enzalutamide in metastatic castration-resistant prostate cancer (CRPC)	Bone scan response	December 2013
Radium-223; Paclitaxel	Phase Ib study of Radium Ra 223 Dichloride in combination with Paclitaxel in cancer subjects with bone lesions	Safety	August 2015

There are also a number early phase clinical trials that have recently published preliminary results utilizing combination therapy. In one Phase I/IIa clinical trial, patients with mCRPC and bone metastases were either given docetaxel alone versus radium-223 plus docetaxel [46]. Initial toxicity results were encouraging, with favorably declines in PSA and ALP favoring the combination group. In another study, an international early access program (EAP) registry trial investigated the effects of concomitant medication on overall survival in mCRPC [47]. In patients receiving radium-223, survival appeared to be better in those treated concomitantly with denosumab or abiraterone.

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Cabazitaxel for the Treatment of Prostate Cancer

12

Michael Kolinsky, Niven Mehra and Johann S. de Bono

Introduction

Prostate cancer is the most common malignancy that affects Western men, and the third most likely to cause their death [1]. While most patients present with localized disease that is amenable to curative intent therapies, as many as one third of these patients will develop metastatic disease, in addition to approximately 15 % who present with de novo metastatic disease [2, 3]. The standard of care for metastatic prostate cancer is androgen deprivation, however after a median of 18–24 months, these patients' cancers will begin to progress despite castrate levels of testosterone, entering a clinical phase referred to as "castration-resistant prostate cancer" or CRPC [4, 5]. Docetaxel was the first treatment that demonstrated an improvement in overall survival in patients with metastatic CRPC [5, 6]. In two pivotal phase III trials, docetaxel showed an overall survival (OS) advantage over mitoxantrone, a chemotherapeutic agent that had previously shown palliative benefits when combined with prednisone, but no improvement in OS over prednisone alone [7]. However, despite more effective systemic therapy, the prognosis for CRPC patients

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remained bleak with a median OS of less than 2 years. However, over the next several years a number of systemic therapies have been introduced that are helping improve outcomes for patients suffering from CRPC. Cabazitaxel represents the first of a new wave of drugs that have demonstrated an OS advantage in metastatic CRPC in the post-docetaxel setting [8]. This drug, which was designed with the goal of overcoming resistance to the first generation taxanes, paclitaxel and docetaxel, has a number of unique properties that make it an appealing weapon in the ongoing battle against CRPC [9–11]. However, concerns have been raised about this drug's toxicities, which include hematologic and gastrointestinal side effects, as well as its high financial cost. Furthermore, ongoing studies are seeking to answer questions regarding the optimal use of this drug in CRPC, such as the most appropriate dose and its use in combinations. In this chapter we will explore the development, pharmacology, and clinical use of cabazitaxel.

Development

The taxane family represents some of the most effective and widely used drugs in cancer treatment. Derived from the precursor molecule 10-deacetylbaccatin-III, paclitaxel and docetaxel are central to curative and palliative treatment paradigms across a number of tumor types; however these drugs, as with other chemotherapies, are limited by the development of resistance [12, 13]. While the clinically relevant mechanisms of resistance are yet to be fully elucidated and will be discussed in detail below, preclinical models have suggested a number of possibilities: Overexpression of members of the ATP-binding cassette family of transporters (P-glycoprotein (P-gp), encoded by the multidrug resistance gene, *ABCB1,* is the best characterized); mutations in tubulin; altered expression of tubulin isoforms, like *TUBB3;* loss of tubulin-stabilizing proteins as well as alterations in proteins associated with cell-cycle checkpoints, DNA damage repair, and apoptosis [3, 14].

Cabazitaxel, another 10-deacetylbaccatin-III derivative, was developed in an aim to identify a taxane derivative that retains the potency of docetaxel in taxane-sensitive cancers, but is capable of overcoming resistance and showing greater potency than docetaxel in taxane-resistant cancers. This drug is structurally very similar to docetaxel, with the presence of methyoxy instead of hydroxyl groups at the C7- and C10-positions (Fig. 12.1) [9]. This results in the appealing properties of a low affinity for P-gp and increased lipophilicity, leading to increased penetration across the blood-brain barrier and possibly cell membranes [9, 14]. Cabazitaxel has demonstrated a broad spectrum of activity in murine and human tumor models, including those with innate resistance to docetaxel due to P-gp overexpression or other mechanisms [14]. Notably, in a model intended to mimic the development of chemotherapy resistance in human cancers, mice bearing xenografts of the murine melanoma cell line, B16 (which is known to be docetaxel sensitive) were made fully docetaxel resistant by a slow process of repeated exposure to docetaxel at the highest non-toxic dose (HNTD). In these mice,

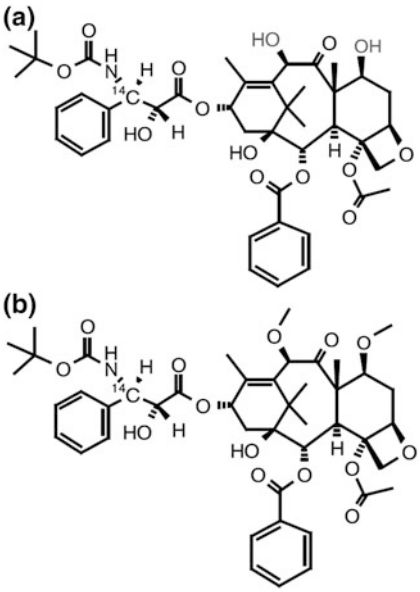


Fig. 12.1 Chemical structure of docetaxel (**a**) and cabazitaxel (**b**). Adapted from Ridoux [94]. With permission from Wolters Kluwer Health, Inc.

cabazitaxel showed similar antitumor activity at the HNTD when compared to the parental B16 docetaxel sensitive tumors (log cell kill[1] 1.3 vs. 2.1 respectively). Interestingly, this tumor model, known as B16/TXT, does not overexpress P-gp, but was rather found to have increased expression of *TUBB3*, which encodes class 3 beta-tubulin, a tubulin isotype that has been implicated in taxane resistance [14].

In prostate cancer models, cabazitaxel has demonstrated significant activity. In mice bearing xenografts of the CRPC cell line, DU-145, cabazitaxel demonstrated complete responses in all 6 animals, and long term tumor free survival in 5 [14]. Also, in mice bearing HID28, another human CRPC tumor model, cabazitaxel showed improved antitumor activity compared to both docetaxel and abiraterone [15].

Mechanism of Action

Microtubules are involved in a diverse range of cellular functions, including cell division, motility, maintenance of cell shape and intracellular transport [16]. They are highly dynamic structures that can easily switch between lengthening and shortening; a tight control is fundamental to maintain all of these cellular functions. The principal mode of action of all taxanes is stabilization of the microtubule cytoskeleton through interaction with β-tubulin, blocking cells in the late G2-M

[1]Log cell kill refers to the logarithmic fractional cell kill, ie 1 log kill is 90 % reduction in tumor cells, 2 log kill is 99 % reduction in tumor cells, etc.

phase of the cell cycle [17, 18]. Control of this cell cycle progression is mitigated by the spindle assembly checkpoint (SAC), which induces mitotic arrest until sister chromatids are anchored correctly to spindle kinetochores and the SAC is satisfied. Yet cells cannot be arrested in mitosis indefinitely: they either die directly in mitotic arrest, or undergo mitotic slippage [19–21]. The determination of cell fate following mitotic slippage is complex and can lead to senescence, apoptosis, necrosis, or adaptation and survival of these cancer cells with genomic aberrations. A p53-dependent pathway of apoptosis is important following taxane-induced mitotic slippage. The post-mitotic G1 checkpoint prevents further DNA reduplication in cells with DNA damage, aneuploidy and non-segregated chromosomes and includes the tumor suppressor proteins p53 and pRb, and the cyclin-dependent kinase inhibitors $p16^{INK4A}$ and $P21^{WAF1}$ [22].

A differential level of microtubule stabilization and distinct downstream effects has been identified between the generations of taxanes. Analyses of C4-2 cells, a CRPC cell line, showed differing gene expression profiles following treatment with docetaxel or cabazitaxel under androgen-deprived conditions: Cabazitaxel treatment showed enrichment in genes involved in the cell cycle and chromosomal organization and regulation, whereas docetaxel appears to have a more significant impact on transcription and repair, putatively caused by differences in microtubule stabilization [23]. Cabazitaxel shows enhanced anti-proliferative and cytotoxic effects compared to docetaxel in chemotherapy-sensitive and resistant cell lines [14] and in a CRPC model [23]. Another reported difference in the mode of action between docetaxel and cabazitaxel might be their extent of inhibition of microtubule-associated transport of full length AR (see section on microtubule-associated transport for more in-depth information). Taxane treatment can inhibit AR activity [24] through disruption of AR nuclear accumulation in pre-clinical models after treatment with both docetaxel and cabazitaxel [25, 26]. Docetaxel has been reported to be cross-resistant with AR-targeting agents, such as enzalutamide, but cabazitaxel remains highly active and exerts its clinical efficacy against enzalutamide resistant/refractory CRPC possibly through AR-independent mechanisms [27, 28]. It has been postulated that inhibition of microtubule-associated transport may be an important mode of action of docetaxel, but not cabazitaxel, however whether this mechanisms of action is relevant in in vivo conditions and in the clinic is unknown.

Mechanisms of Resistance

Altered Transporters and Metabolism

Cabazitaxel was developed based on its activity against docetaxel-resistant tumor models as a poor substrate for P-gp [14]. Even though a poor substrate, cabazitaxel still displays an approximate 10-fold resistance to high P-gp expressing cell lines and its sensitivity can be completely restored by the addition of a P-gp inhibitor

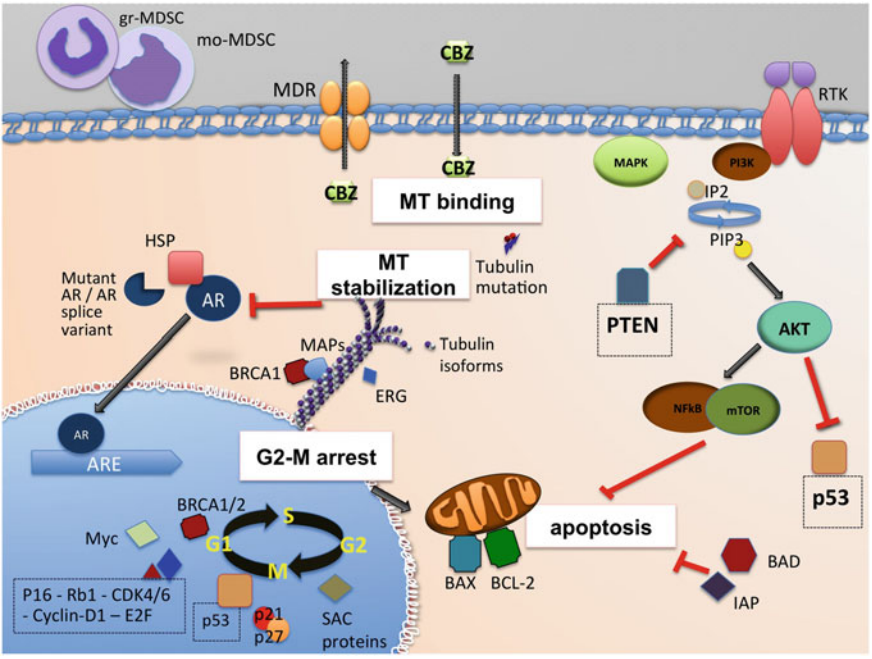


Fig. 12.2 The intracellular concentration of cabazitaxel (CBZ) is an important determinant of cellular outcome with low intracellular concentrations driving resistance. Other mechanisms of resistance, are tubulin mutations and enhanced microtubule dynamicity that hamper effective CBZ binding and MT stabilization. Any aberration decreasing the stringency of G2/M control may lead to increased mitotic slippage; concurrent loss of post-mitotic checkpoint control in the presence of genomic aberrations increase tumor cell adaption, survival and resistance. An altered apoptotic response through differential expression in pro- and anti-apoptotic proteins (e.g. by enhanced signaling of pro-survival pathways) or mutations or loss of key apoptotic proteins also contributes to CBZ resistance. Additional mechanisms of resistance depicted are constitutive AR signaling through mutant AR or AR splice variants, and by modulation of the tumor microenvironment (e.g. change in extracellular matrix proteins and presence of MDSCs)

[29]. ABCC2 (MRP2) was shown to transport taxanes [30], however no activation of MDR transporters, such as ABCC2, ABCC10 (MRP7) or ABCG2 (BCRP) was identified as a resistance mechanism in cabazitaxel-resistant cell lines [29]. A dependent of cellular fate is the intracellular concentration of a taxane and a lower intracellular concentration of drug does not trigger a sustained mitotic arrest but leads to a mitotic delay, mitotic slippage and survival [31]. Survival of cells with aneuploidy and a chromosomal instability phenotype are associated with taxane resistance [32, 33]. Possible additional mechanisms that decrease intracellular concentration are alterations in cellular permeability, cellular importers, and via polymorphisms in CYP3A4, CYP3A5 and CYP2C8 (Fig. 12.2) [34].

Altered Microtubule Binding and Microtubule Dynamics

The dynamicity of microtubules affects taxane binding, which is influenced by tubulin isoform expression, mutation, post-translational modification, and altered binding or regulation of microtubule associated proteins (MAPs). Six β-tubulin-isotypes have been described with tissue specific or constitutive expression [35]; βIII-tubulin exhibits more microtubule dynamicity than other isoforms and associates with resistance to the first-generation taxanes docetaxel and paclitaxel [16, 36, 37]. Reports on βIII-tubulin and cabazitaxel resistance are somewhat conflicting: In the pivotal melanoma cell line B16 with acquired docetaxel-resistance used for the selection of cabazitaxel, the drug retained its activity whilst having three-fold increased expression of βIII-tubulin [14]; however, studies using the breast cancer MCF-7 cell-line with acquired cabazitaxel resistance identified altered tubulin dynamicity with increased βIII-tubulin levels as a putative mechanism of resistance [29]. Altered expression patterns of tubulin isotypes such as βIV- and α-tubulin have to date only been connected with sensitivity to first generation taxanes. Mutations in β-tubulin that impact taxane-microtubule binding have been identified in the N-terminal and central domain in cell lines [16, 38] and in patients with non-small cell lung cancer [39]. There is no evidence connecting tubulin mutations or polymorphisms with cabazitaxel sensitivity in vivo. Post-translational modification of the variable C-terminal region of tubulin additionally influences microtubule dynamics by specifically changing its affinity for several MAPs and motor proteins [16]. MAPs that influence microtubule dynamicity have only been associated with first generation taxane sensitivity, and have been reviewed elsewhere, and include Tau and the MAP destabilizing protein Stathmin. The ERG transcription factor has also been identified as a MAP that predominantly interacts with β-tubulin [40] and enhances microtubule dynamicity. In a small cohort of 34 patients, patients with ERG-positive tumors had an almost 2-fold lower rate of PSA-response than patients with ERG-negative tumors when treated with docetaxel [13]. Cytoplasmic BRCA1 indirectly regulates microtubule stability, likely mitigated through regulation of MAPs and possibly through tubulin dimer ubiquitination. The absence (or low expression) of BRCA1 has been reported to lead to more microtubule dynamicity and less sensitivity to taxane-induced stabilization and interphase cell death [41]. BRCA1 expression has also been associated with taxane resistance in ovarian, breast, bladder, and lung cancer [42–45] but not in patients with prostate cancer. BRCA germline mutations were not, however, associated with resistance in CRPC models [46]; however, BRCA1 protein expression and loss of cytoplasmic function in these mutants was not reported on. In two cabazitaxel-resistant cell lines with ABCB1-dependent and independent resistance mechanisms, both MCF-7 cell lines demonstrated a decreased BRCA1 protein expression. Following treatment of the sensitive parental MCF-7 cell line with a pool of siRNAs against BRCA1, these cells developed an approximately 4-fold resistance to cabazitaxel [29].

Altered Checkpoint and DNA Repair Genes

Many of the SAC proteins have been implicated with first generation taxane sensitivity and are reviewed elsewhere. Aberrations in these SAC proteins are known to increase aneuploidy and chromosomal instability, and are more resistant to mitotic arrest induced by microtubule inhibition [47]. DNA repair genes associated with taxane resistance include BRCA1 [29, 41], Fanconi anemia group F (FANCF) and ERCC1 [29]. Altered post-mitotic checkpoints are common aberrations in prostate cancer; p53 is commonly mutated or lost, as is pRb1 which is aberrant in approximately 21 % of patients with metastatic CRPC [48]. Functional p53 status is reported to be a determinant of docetaxel sensitivity in prostate cancer cell lines [49, 50]. Taxane-induced phosphorylation of 15Leu on p53 was shown to be required for p53-dependent apoptosis [50]. The functional relevance of p53 aberrations and its regulator MDM2 in relation to cabazitaxel sensitivity has yet to be determined. Rb1 is another critical regulator of cell cycle progression through the repression of E2F transcription factors which activate genes required for DNA replication, nucleotide synthesis and checkpoint control [51]. There is important emerging evidence that Rb1 loss is associated with docetaxel, paclitaxel [23, 52–54] and cabazitaxel sensitivity [23] by sensitizing cells to p53 dependent- and independent apoptosis. Whilst functional p53 and aberrant Rb1 pathway likely enhances sensitivity to cabazitaxel, a combined inactivation of both post-mitotic checkpoint regulators is reported to associate with a taxane resistant phenotype [55]. Clinical studies now need to interrogate these questions in large patient numbers to elucidate whether these reports are clinically relevant.

Inhibition of Microtubule-Associated Transport

Many key proteins including the androgen receptor (AR) are transported over microtubules, and disruption of this trafficking could affect cellular signalling and function [56]. A link between the clinical activity of taxanes and AR signalling was postulated, as inhibition of AR nuclear accumulation was seen in cell lines treated with taxanes and in selected circulating tumor cells (CTCs) from patients [25]. Due to observed cross-resistance between androgen-targeting agents and first generation taxanes in vitro [28] and in vivo [57, 58], it was thought that AR mutations or splice variants that do not require microtubule-associated transport [in particular AR splice variant 7 (ARv7)], could contribute to taxane resistance [59]. In contrast to docetaxel, there appears to be no cross-resistance with androgen-targeting agents seen with cabazitaxel [27] and pre-clinical studies suggest that cabazitaxel exerts most if its antitumor activity via AR-independent mechanisms [28]. However, recent reports question whether inhibition of AR-transport is a true mode of action of taxanes in vivo: first, supra-pharmacological concentrations of taxanes were used in all the pre-clinical models, whilst at nanomolar concentrations, taxanes do not appear to inhibit dynein-mediated transport [23]; second, in recent clinical studies no association of ARv7 and primary resistance to taxanes was demonstrated

[60, 61]. Active AR signaling may therefore drive resistance to taxanes through downstream signaling and activation of pro-survival pathways, but ARv7 expression does not seem to play any role in the induction of cabazitaxel resistance.

Altered Apoptotic Response

The apoptotic response following taxane chemotherapy signals through both the intrinsic (or mitochondrial) and extrinsic (or death-receptor) pathways [62]. The intrinsic pathway can be deregulated by inhibitor of apoptosis proteins (IAP), an altered balance of Bcl2 apoptotic proteins, or by an altered phosphorylation status of Bcl2. In the MCF-7 cell line that was made cabazitaxel-resistant, decreased expression of the anti-apoptotic regulators Bcl2, MCL1, and several IAPs was seen [29]. In prostate cancer many cellular stress response pathways have been found to be deregulated [48]. Aberrations in these pathways associate with taxane sensitivity, and include PI3K/AKT [63, 64], MAPK/ERK [65], TNF [66], NOTCH [67, 68], Jak2/Stat3 [69] and NF-kB [70]. Other pathways implicated with taxane resistance are activation of IGF, through signalling via the IGF-axis and downstream activation of kinase pathways, including PI3K, JNK and MAPK [71]. Clusterin is another important mediator of the stress response, and may confer cabazitaxel resistance by suppressing stress-induced apoptosis [72]. Lastly, the heat-shock proteins (HSP) 27, 70 and 90, are molecular chaperones interacting with key proteins inducing a pro-survival phenotype and have been all been implicated with taxane sensitivity [73–75].

Epithelial-to-Mesenchymal Transition

Treatment with taxanes has shown to induce an epithelial-to-mesenchymal transition that may induce a resistance phenotype [76]. Gene expression profiling in cabazitaxel-resistant cell lines demonstrated increased expression of Vimentin (VIM) and decreased expression of E-cadherin (CDH1), a mesenchymal and epithelial marker, respectively [29]. MiR-200 family members are known to induce an EMT phenotype [77] and to induce docetaxel resistance [76, 78, 79].

Myeloid-Derived Suppressor Cells

The tumor environment attracts and expands immature myeloid cells into so-called myeloid-derived suppressor cells (MDSCs) with immunosuppressive characteristics. These cells control senescence evasion and chemoresistance in a PTEN-null model; inhibition of recruitment of MDSCs by chemokine inhibitors restores docetaxel sensitivity. Their detection in peripheral blood and in the tumor microenvironment associates with poorer outcome and resistance to first-generation taxanes [80]. Activation of the Jak2/Stat3 pathway may induce this strong immunosuppressive

environment, and decreasing Jak2/Stat3 signalling by JAK2 inhibitors can restore immune surveillance and enhance taxane chemotherapy efficacy [69].

Additional Genes Associated with Taxane Sensitivity

Other genes associated with cabazitaxel resistance are genes associated with neuroendocrine differentiation, such as N-MYC and Aurora-kinase A [81]; detoxification such as glutathione S-transferase P1 (GSTP1) and gluthathione peroxidase 3 (GPX3) [29]; the actin cytoskeleton [35, 82]; the extracellular matrix [83].

MicroRNAs

MicroRNAs (MiRs) are small non-coding RNAs that post-transcriptionally regulate gene expression through translational repression or degradation of target mRNAs. MiRs have been reported to be deregulated in prostate cancer [77]. Many MiRs have been implicated with taxane resistance and include MiR-130a, MiR-301a, MiR-181a [84], MiR-34a, MiR-148a [85, 86], MiR-135a [87], MiR-21 [88], MiR-205 [78] and MiR-17 and MiR-200 family members [79].

Pharmacokinetics

The human pharmacokinetic (PK) data for cabazitaxel is informed by three phase I trials (one of which used weekly dosing), a phase II trial in breast cancer, and data from 67 patients from the phase III TROPIC trial in metastatic CRPC [8, 10, 11, 89, 90]. Furthermore, Ferron and colleagues performed a population PK analysis using the combined data from these studies [91]. Cabazitaxel has a similar PK profile to docetaxel, with patients treated every 3 weeks showing dose-proportional exposure and triphasic elimination. Cabazitaxel appears to have a deeper peripheral compartment, which results in a very large steady state volume of distribution (Vss) and a very long elimination half-life (Table 12.1) [9]. Interestingly, the clearance of cabazitaxel was found to be approximately 60 % lower in breast cancer patients compared to patients with other tumor types [91]. Given that most of the breast cancer patients (34 of 37) were from a single study [90], it is possible that this difference is due to study effect, rather than a true pharmacokinetic difference in breast cancer patients, which is further supported by the fact that gender did not influence inter-individual variability in clearance. Furthermore, a phase I/II study that treated breast cancer patients with the combination of cabazitaxel and capecitabine, found a clearance of cabazitaxel of 33.6 L/h/m^2, which is more in keeping with data from other solid tumors, with capecitabine having no apparent effect on the PK of cabazitaxel [92]. The only other factor that associated with clearance was body surface area (BSA), with larger patients demonstrating higher clearance, which justifies BSA based dosing. There is no evidence of accumulation or changes

Table 12.1 PK parameters for cabazitaxel in non-breast solid tumors

Clearance	Central volume of distribution	Vss	Half life alpha	Half life beta	Half life gamma
48.5 L/h	26.0 L	4870 L	4.4 min	1.6 h	95 h

Only data from non-breast cancer patients is included, as the data from breast cancer patients shows a significant difference in clearance when compared to other solid tumors, and is likely due to study effect as opposed to a true difference between tumor types
Based on data from Ref. [91]

in PK parameters after up to three cycles of treatment given every 3 weeks [91]. However, in patients treated with weekly dosing for 4 out of every 5 weeks, drug accumulation was seen with a significant increase in exposure as measured by AUC after day 22 dosing [89]. Of interest, abnormalities in renal or hepatic function did not significantly alter the PK of cabazitaxel. However, these results need to be interpreted with caution: Only one patient with a creatinine clearance of <30 mL/min, one with elevated bilirubin, and relatively few with elevations in transaminases or alkaline phosphatase were included in these studies, making it difficult to draw conclusions in this population [91, 93].

Cabazitaxel is predominately metabolized through the liver with in vitro experiments showing 80–90 % of the drug being metabolized by CYP3A4/5, with CYP2C8 being responsible for the remainder [93]. Mass balance studies using radiolabelled cabazitaxel show that the kidneys contribute very little to the excretion of the drug, with 3.7 % of recovered radioactivity detected in the urine [94]. Analysis of the metabolism of cabazitaxel has revealed 19 metabolites generated through four pathways: 10-*O*-demethylation; 7-*O*-demethylation; hydroxylation of the *t*-butyl moiety of the lateral chain; and cleavage of the taxane ring from the lateral chain (Fig. 12.3a, b). Cabazitaxel is the main circulating compound, with 7 metabolites being detected in the plasma, each representing <10 % of the total AUC. Of note, docetaxel is formed by demethylation of the 7-*O* and 10-*O* groups, and can be detected in the plasma of some patients, where it represents 3–4 % of total drug exposure [94].

An interesting property of cabazitaxel is that it to penetrates into the CNS better than first generation taxanes. The vasculature in the brain contains tight junctions between endothelial cells, which also express P-gp as well as other efflux pumps, forming the blood brain barrier (BBB), which serves to protect the brain from toxic insults [95]. The improved penetrance into the brain by cabazitaxel is felt to be due to the fact that it is more lipophilic and a weaker substrate of P-gp compared to docetaxel and paclitaxel [9, 96]. Studies in mice show that cabazitaxel reaches its maximal concentration in the brain at 15 min after intravenous infusion, with exposure as measured by $AUC_{0–48\ h}$ nearly 4 fold higher in the brain compared to plasma due to slower clearance. Furthermore, using ^{14}C-cabazitaxel infusions in mice, rats, and dogs, a consistent relationship between radioactivity exposure in the brain and blood is seen across species, suggesting that a similar relationship may be seen in humans [96]. From a clinical perspective, this CNS penetrance raises two issues: activity against brain metastases; and central neurotoxicity. Brain metastases

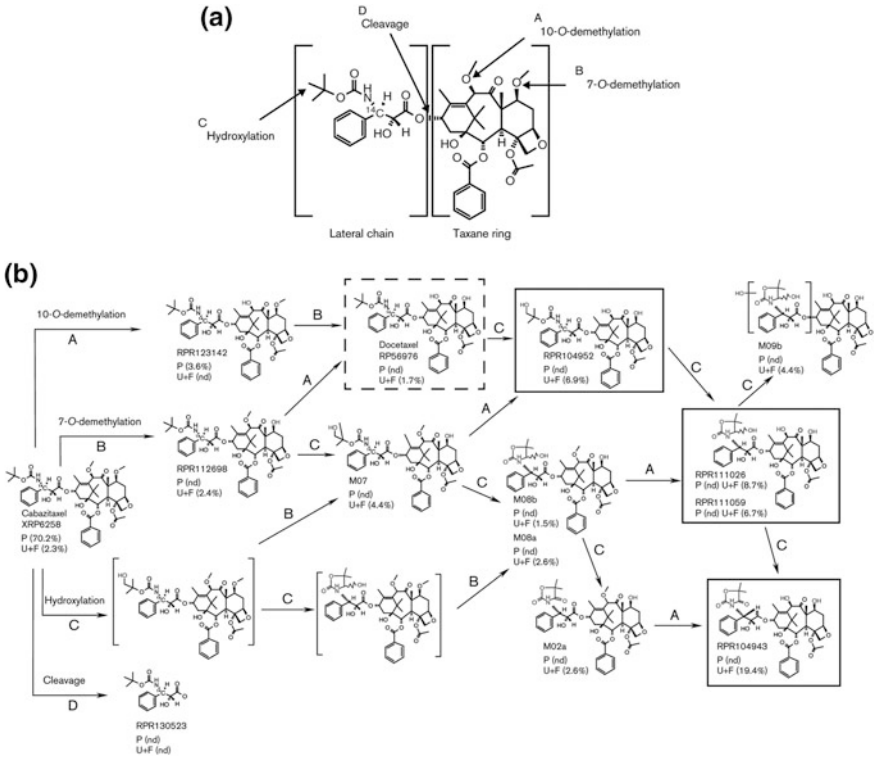


Fig. 12.3 **a** Proposed schematic of the principal metabolic pathways of cabazitaxel. **b** Proposed outline of the metabolic pathways of cabazitaxel and structure of the main metabolites. *F* feces; *nd* not detected; *P* plasma; *U* urine; [ ], intermediate not detected; square box, metabolic pathway of docetaxel. P (%) represents the mean percent of plasma radioactivity AUC; U + F (%) represents the mean percent of the dose excreted in urine and feces

in prostate cancer are rare, with modern series of CRPC patients showing an incidence of approximately 2–3 % [97, 98]. Also, the relevance of the BBB in patients with clinically apparent brain metastases is unclear, given the fact that brain metastases disrupt the BBB by generating abnormal blood vessels with significantly increased permeability compared to the normal, intact BBB, and also most patients with brain metastases receive radiotherapy, which further disrupts the BBB [95]. Nonetheless, De Placido and colleagues report on 3 patients with metastatic CRPC with brain metastases treated with cabazitaxel in addition to whole brain radiotherapy. All three patients showed a response in the brain metastases, including one complete response [99]. Regarding the possibility of central neurotoxicity, degenerative lesions were seen in mice treated with cabazitaxel, but not in rats or dogs. However, similar changes have also been demonstrated in mice treated with paclitaxel, and clinically this doesn't appear a concern as central neurotoxicity wasn't reported in the TROPIC patients, nor in the patients treated in the expanded access programs [8, 9, 100–105].

Clinical Activity

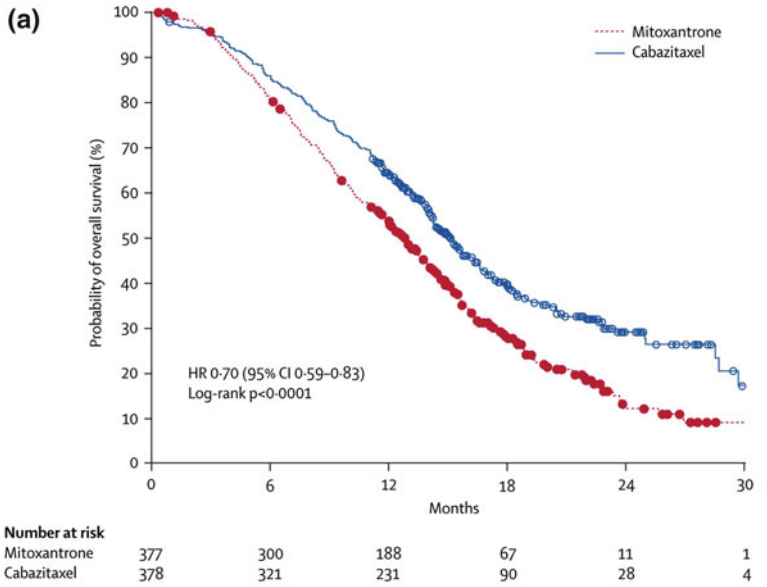
In the phase I trials that delivered cabazitaxel as a one-hour intravenous infusion every 3 weeks performed by Mita and Dieras, the maximum tolerated dose (MTD) reported were 25 and 30 mg/m^2, respectively. The design of these trials differed in that Mita and colleagues performed a traditional 3 + 3 design, with dose escalation based on the toxicities seen in each cohort, whereas the Dieras trial was based on intrapatient dose escalation, with the dose of subsequent cycles based on the toxicity seen in the previous cycle. It is important to note that in the Dieras trial, 3 of 7 patients treated at the 20 mg/m^2 dose at the first cycle had a dose limiting toxicity (DLT), which met the predefined criteria for the MTD. However, because two of these DLT's were grade 3 diarrhea that quickly improved with supportive treatment with loperamide, dose escalation was allowed. Subsequently 30 mg/m^2 was found to be the MTD based on hematologic DLT's in three of five patients, and therefore dose de-escalation to 25 mg/m^2 was performed, with only one of six patients experiencing a DLT in the first cycle at this dose level, and was thus the recommended phase II dose found in this study [10, 11]. A phase II trial in breast cancer patients also informed the recommended dose selection of cabazitaxel. This trial used an initial dose of 20 mg/m^2 at the first cycle, with escalation to 25 mg/m^2 at the second cycle if no severe toxicities were encountered. Dose escalation was performed in 20 of 71 patients with no subsequent increase in adverse events [90]. The Dieras trial did not include any patients with CRPC, while the Mita trial included 8, with two obtaining objective partial responses, and a third having a minor response, not meeting formal criteria for a partial response.

Based on this data, the pivotal phase III TROPIC trial was initiated in patients with metastatic CRPC, with a chosen dose of 25 mg/m^2. This trial aimed to answer the question of whether cabazitaxel in combination with daily prednisone would improve survival over mitoxantrone and prednisone, in patients with metastatic CRPC who had progressive disease during or after docetaxel chemotherapy [8]. Patients were randomized to receive either cabazitaxel given as a 1-h intravenous infusion, or mitoxantrone given intravenously over 15–30 min, both in combination with prednisone 10 mg daily. For patients in the mitoxantrone group, cross-over to cabazitaxel at progression was not allowed. Treatment was continued for up to 10 cycles in order to minimize the risk of cumulative cardiotoxicity that can be seen with mitoxantrone, which is a member of the anthracenedione antineoplastics [106]. Prophylactic GCSF was not allowed with the first cycle, but could be used for subsequent cycles for prolonged or complicated neutropenia, at the treating physician's discretion. Of note, weekly monitoring of blood counts was performed during each cycle, in addition to when it was clinically indicated. Randomization was successful in that the two treatment arms were well balanced with no significant differences in baseline characteristics. Importantly, two thirds of patients in both arms had progressed either during (approximately 30 %) or within three months (approximately 45 %) of docetaxel chemotherapy, indicating a highly docetaxel-resistant population.

The primary endpoint of improved overall survival was met, with median OS of 15.1 months in the cabazitaxel arm, compared to 12.7 months in the mitoxantrone arm, with a corresponding hazard ratio of 0.70 for risk of death (Fig. 12.4a) ($p < 0.0001$). This benefit was seen across all subgroups, including those patients who had progressed during docetaxel, though not all subgroups reached statistical significance (Fig. 12.4b). An updated analysis showed that patients in the cabazitaxel arm were twice as likely to be alive at 2-years, with a probability of surviving longer than 2-years of 27 % compared to 16 % in the mitoxantrone arm ($p < 0.0001$). This analysis showed factors associated with survival longer than two years were treatment with cabazitaxel, rising PSA at baseline, and longer time from first hormonal treatment to enrolment in TROPIC; whereas elevated ALP or pain at baseline, and less than 6 months from the last dose of docetaxel to randomization in TROPIC were associated with survival of less than 2 years [107]. All secondary endpoints favored the cabazitaxel arm, with the exception of pain response rate and time to pain progression, which were similar between the two arms. There was also no significant difference in changes in PS, or time to PS deterioration [107]. In patients with measurable disease, objective responses were seen in 14.4 %, with ≥50 % PSA responses in 39.2 % of patients treated with cabazitaxel; compared to 4.4 % ($p = 0.0005$) and 17.8 % ($p = 0.0002$) in the mitoxantrone group. The median time to tumor progression was 8.8 months compared to 5.4 months in the mitoxantrone group ($p < 0.0001$). Patients in the cabazitaxel arm received more cycles than those receiving mitoxantrone, with a median of 6 compared to 4, and more than twice as many patients completing the planned 10 cycles of treatment; however, patients receiving cabazitaxel were more likely have dose reductions or treatment delays.

Safety

Overall hematologic toxicity was similar between the two arms in the TROPIC trial; however, patients who received cabazitaxel had significantly higher rates of severe hematologic toxicity, with febrile neutropenia occurring in 8 %, including 7 patients who died as a result of neutropenic complications. The rates of neutropenia differed by geographic region, with the lowest rates in Europe, followed by North America, and with patients from other regions having the highest rates. Also, patients 65 years of age or older had higher rates of neutropenia compared to younger patients. Non-hematologic toxicity was generally increased in the cabazitaxel arm with higher rates of diarrhea, fatigue, asthenia, abdominal pain, dyspnea, cough, nausea and vomiting, dysgeusia, hematuria, urinary tract infections, pyrexia, alopecia, and peripheral neuropathy compared to patients receiving mitoxantrone, with most of these toxicities being grade 1 or 2. The most common reasons for discontinuing treatment due to adverse reactions were neutropenia and renal failure. In addition to the toxicities seen in the trial, there have been post-marketing reports of significant gastrointestinal adverse events including colitis, gastritis, hemorrhage, perforation, ileus, and obstruction. Hypersensitivity reactions appear to be uncommon, likely due to prophylaxis with steroids and antihistamines; however,



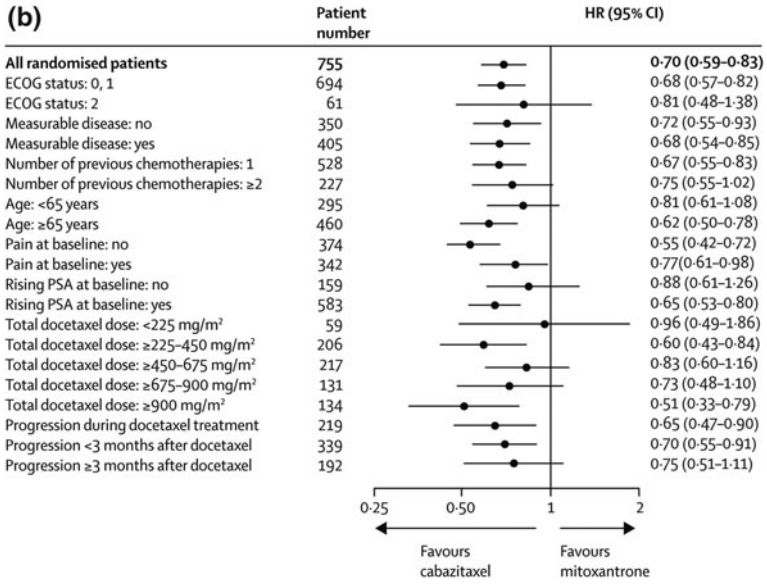


Fig. 12.4 Overall survival of patients in the pivotal phase III TROPIC trial [8]. **a** Kaplan-Meier estimates of the probability of survival in all patients randomly assigned to treatment with cabazitaxel plus prednisone or mitoxantrone plus prednisolone. The points on the curve show censored observations. **b** Intention-to-treat analysis of overall survival in subgroups of patients defined by baseline characteristics. Hazard ratios (HR) lower than 1 favour the cabazitaxel group and greater than 1 favour the mitoxantrone group. With permission from Elsevier

because cabazitaxel is formulated with polysorbate-80, patients who have had severe hypersensitivity reactions to docetaxel or other drugs formulated with polysorbate-80 should not be treated with cabazitaxel [108].

Of concern, patients receiving cabazitaxel had a higher rate of death within 30 days of last treatment, with 5 % compared to 2 % for mitoxantrone. Nearly half of these deaths were due to complications of neutropenia, and as such were treatment related. In addition, 5 fatal cardiac events were recorded, with none seen in the mitoxantrone arm. Interestingly, however, mortality was low in patients treated in North America at 0.3 %, 3 % in patients treated in Western Europe and highest in patients treated in the rest of the world.

After US Food and Drug Administration approval of cabazitaxel for second line treatment of metastatic CRPC a number of groups published their experience with cabazitaxel in patients treated in expanded-access programs [100–105]. These publications show generally lower rates of toxicity than what was seen in TROPIC. For instance, Heidenreich and colleagues published the experience of the European expanded access program, which enrolled 746 patients from 20 European countries. Eligibility criteria for this program were similar to TROPIC and indeed the baseline characteristics of these patients were much the same; however, unlike TROPIC, primary prophylaxis with GCSF was permitted, using recommendations from American Society of Clinical Oncology as guidance [109]. In this group, 43 % of patients received GCSF as primary prophylaxis during the first cycle, with a total of 55.1 % receiving prophylactic GCSF at any cycle. As may be expected with such high utilization of prophylactic GCSF, the rates of neutropenia were much lower than in TROPIC, at 19.8 % for all grades, and 17.5 % for grade 3 or higher. Furthermore, the rate of febrile neutropenia was lower, at 5.5 %. In elderly patients, the risk of neutropenia and its consequences were higher, but not dramatically so, likely due to increased use of GCSF. The factors associated with the greatest risk of severe and/or complicated neutropenia in descending order of association were: first cycle, neutrophil count <4, and age 75 or older. The only factor associated with a decreased risk was G-CSF prophylaxis with an odds ratio of 0.70, indicating a 30 % reduction in the risk of severe or complicated neutropenia ($p = 0.04$) [100]. It is worthwhile noting that in the patients receiving GCSF at the first cycle, the risk of grade 3/4 neutropenia is actually similar to patients who did not receive prophylactic GCSF, at approximately 8 %; however, the patients receiving GCSF likely had a higher initial risk [101]. Heidenreich also report lower rates of diarrhea with 2.8 % experiencing grade 3/4 diarrhea, compared to 6 % in TROPIC. These results are echoed in the individual publications of the German, UK, Spanish, Italian, and Dutch expanded access programs (Table 12.2). What these publications tell us is that the toxicities experienced in a "real world" setting may be lower than what is described in TROPIC, likely due to an increased awareness of the expected toxicities and providing proactive supportive care, such as GCSF prophylaxis and anti-motility agents.

Regarding other toxicities, cabazitaxel appears to have a somewhat favorable profile compared to docetaxel. Omlin compared the new or worsening adverse events seen in the 371 patients treated with cabazitaxel in the TROPIC trial and compared these to the combined 930 patients treated with docetaxel in the phase 3

Table 12.2 Data from European expanded access programs

Expanded access program	Number of patients	Grade 3/4 neutropenia (%)	Grade 3/4 diarrhea (%)	Possibly treatment related deaths (%)	Prophylactic GCSF use (first cycle + subsequent cycles)
Italian [105]	218	33.9	2.8	1.8	62.4 % total
Spanish [104]	153	16.3	5.2	3.3	57.7 + 12.4 %
UK [101]	112	9.8	4.5	3.6	79.5 + 5.3 %
German [103]	111	7.2	0.9	3.6	13.5 + 3.6 %
Dutch [102]	51	4.1	2.0	0.0	16.3 % total
European [100]	746	17	2.8	0.9	43 + 12.1 %
TROPIC [8]	378	82	6	5	Not reported

trials TAX327 and VENICE. The patients treated with cabazitaxel showed significantly lower rates of alopecia, nail changes, neuropathy and dysgeusia (Table 12.3). Furthermore, grade 3–4 peripheral neuropathy was rare in patients

Table 12.3 Percentage of patients who had new or worsening AEs. Adapted from Omlin et al. [110]

Grouped AE term	DOC Comb, n = 930 T, n = 332 V, n = 598		CAB TROPIC, n = 371		*P* (Comb DOC vs. CAB) Odds ratio (95 % CI)
Percentage all grades	Percentage grades 3–4	Percentage all grades	Percentage all grades	Percentage grades 3–4	
Alopecia	Comb: 52.2 T: 65.4 V: 44.8	NA	10	NA	<0.0001 9.84 (6.84–14.2)
Nail changes	Comb: 27 T: 29.8 V: 25.6	0	3.5	0	<0.0001 10.2 (5.78–18.3)
Neuropathy	Comb: 40.8 T: 37.7 V: 42.5	Comb: 3.2 T: 2.1 V: 3.8	27.8	1.1	<0.0001 1.79 (1.38–2.33)
Dysgeusia	Comb: 19.5 T: 18.4 V: 20.1	0	11.1	0	0.0003 1.95 (1.35–2.80)
Neutropenia	Comb: 34 T: 41 V: 31	Comb: 25 T: 32 V: 22	94	82	<0.0001 0.035 (0.023–0.055)
Febrile neutropenia	NA	Comb: 4 T: 3 V: 4	NA	8	0.0041 0.48 (0.29–0.80)
Death within 30 days of last dose of chemotherapy	NA	Comb: 4 T: 3 V: 4	NA	5	0.3201 0.74 (0.41–1.34)

AE Adverse event; *CAB* cabazitaxel; *Comb* combined; *T* TAX327 trial database; *V* VENICE trial database

treated with cabazitaxel, occurring in only 1 % [110]. This is also supported by the expanded access programs, which confirm peripheral neuropathy and nail changes are rare [104, 105].

Prognostic Factors

While the TROPIC trial demonstrated at least a trend towards benefit in all subgroups, a number of studies have investigated prognostic factors for patients receiving cabazitaxel. Halabi and colleagues developed a prognostic model for patients receiving second line chemotherapy using the TROPIC data for testing and training, and data from the SPARC trial, a trial of sartraplatin versus placebo in post docetaxel patients, for validation. This model found several prognostic factors: pain, measurable disease, ECOG performance status, progression on docetaxel within 6 months, visceral disease, duration of hormonal therapy, hemoglobin, PSA, and alkaline phosphatase, with progression on docetaxel within 6 months and ECOG performance status being the most important. This model, which uses clinically available prognostic factors is capable of stratifying patients into low, intermediate, and high groups, which in the testing set of patients from the TROPIC trial showed respective median OS of 8.3, 14.9 and 23.7 months ($p < 0.001$) [111].

In addition to this model, baseline neutrophil-to-lymphocyte ratio has been found to be associated with PSA response, RECIST response, and overall survival in the TROPIC patients, independent of the prognostic factors found by Halabi, treatment arm, and baseline corticosteroid use [112]. For patients with a baseline NLR ≥3 compared to those <3, the hazard ratio for OS was 1.55 ($p < 0.001$). Furthermore, conversion from a high (≥3) to low (<3) NLR was associated with a significant improvement in overall survival, with a hazard ratio of 0.66 ($p = 0.001$), compared to those who maintained a high NLR. While NLR is associated with survival across different treatments and indeed different disease types, this was the first study in CRPC that showed the significance of the NLR remained regardless of baseline corticosteroid use, which is an important consideration given the immunosuppressive effects of these drugs.

Furthermore, a small Italian series of 47 patients identified Gleason score of ≥8 to be associated with a prolonged PFS, but not OS on multivariate analysis. Not surprisingly, this analysis also identified patients with visceral metastases as being associated with shorter PFS and OS [113]. However, it is important to note that both of these subcategories of aggressive disease still benefit from cabazitaxel relative to mitoxantrone, as demonstrated in a post-hoc analysis of the TROPIC data [114].

Monitoring Response to Treatment

Monitoring PSA has been an important component of assessing response in patients treated with systemic therapy for CRPC, as reductions in PSA correlate with improved survival [115, 116]. In the two first-line docetaxel phase III trials,

TAX327 and SWOG 99-16, a PSA fall of ≥30 % within 3 months of treatment was found to satisfy the Prentice criteria as a surrogate marker for overall survival for patients receiving first line chemotherapy. While the degree of surrogacy was high in the analysis of SWOG 99-16, the analysis of the TAX327 trial demonstrated that the degree of surrogacy as measured by the proportion of treatment effect was found to have very wide confidence intervals, suggesting that the degree of surrogacy was modest, leading the authors to conclude that OS remains the preferred endpoint in CRPC trials [117, 118].

Halabi and colleagues used the data from the TROPIC trial to investigate whether PSA fall of either ≥30 or ≥50 % within 3 months of treatment is a surrogate marker for overall survival for patients treated with second line chemotherapy. They used a number of analytic techniques to examine this including the Prentice criteria, proportion of treatment effect explained, and a meta-analytic approach that allows for the assessment of surrogacy at the individual patient level as well as the trial level. While both PSA response criteria were significantly associated with improved overall survival compared to patients who did not have a PSA response, criteria for surrogacy was not met at either the individual patient or trial level for either response criteria. These results indicate that while PSA responses are important and can provide prognostic information, they cannot be used as a surrogate endpoint for OS [116]. Furthermore, like docetaxel, a PSA "flare" phenomenon has been observed with cabazitaxel, where an initial rise in PSA is followed by a decline. Depending on the definition used, 8–31 % of patients treated with cabazitaxel will have such a PSA "flare" lasting up to 10 weeks, with these patients having similar PFS and OS compared to patients who respond immediately, and showing significantly better outcomes than patients with no PSA decline [119]. These findings further confirm that PSA alone, particularly early changes within the first 12 weeks should not prompt discontinuation of treatment, in accordance to the recommendations made by PCWG2, as PSA kinetics don't adequately explain the activity of cabazitaxel [116, 120].

Controversies and Unanswered Questions

While cabazitaxel was the first systemic therapy to show an OS benefit in the second line setting in metastatic CRPC, since this approval a number of other agents have also demonstrated OS benefits, including abiraterone and enzalutamide. Because of this, many patients are receiving cabazitaxel in the third or even fourth line. An important question is whether prior treatments impact the efficacy of cabazitaxel. Because TROPIC was initiated prior to the availability of abiraterone and enzalutamide, this study did not include patients previously treated with these drugs. As discussed above, there is some evidence of cross resistance between these agents and docetaxel, with reports suggesting that docetaxel may be less active in patients who have received abiraterone [56, 57, 59, 121, 122]. This does not appear to be the case with cabazitaxel: Al Nakouzi and colleagues report on 79 patients treated with cabazitaxel after docetaxel and abiraterone and show similar outcomes

to what was seen in TROPIC, with smaller series from Pezaro and Sella showing similar results [27, 28, 97, 123]. Furthermore, prior response to abiraterone doesn't appear to predict response to future cabazitaxel [97]. Also, in an analysis of 350 CRPC who received docetaxel as initial treatment, those who subsequently received both cabazitaxel and abiraterone had an improved OS compared to those who received just one of these agents [124]. While caution is required when interpreting small retrospective studies, these results do suggest that cabazitaxel is not cross-resistant to abiraterone and enzalutamide, and provide further support to the evidence that cabazitaxel may act differently to docetaxel in CRPC.

Another important question is whether cabazitaxel is most efficacious in the post-docetaxel space, or whether it should be used up-front in place of docetaxel as a first line chemotherapy. Preclinical data suggests that cabazitaxel may be more active than docetaxel in treatment naive CRPC cancers [15, 23], and this hypothesis is being tested in the FIRSTANA trial (ClinicalTrials.gov identifier: NCT01308567), where chemotherapy naive metastatic CRPC patients will be randomized to receive either docetaxel at 75 mg/m^2, or cabazitaxel at either 25 or 20 mg/m^2. While this trial will provide valuable information, given the results of the CHAARTED and STAMPEDE trials that show an overall survival benefit in the hormone sensitive population, it is unclear how this will affect the use and efficacy of cabazitaxel, as FIRSTANA will not address this question. Moreover, all patients randomized to docetaxel on FIRSTANA will have the option of receiving later cabazitaxel making it highly unlikely that this trial will show a survival advantage.

As mentioned previously, the optimal dose of cabazitaxel is also somewhat in question, with discrepancies in the recommended phase II dose of the two phase I trials, and with the high rates of toxicity seen in TROPIC at 25 mg/m^2. Two trials are poised to answer the question whether the efficacy of cabazitaxel can be maintained at 20 mg/m^2, while reducing toxicity: FIRSTANA will address this in the chemotherapy naive population; and PROSELICA (ClinicalTrials.gov identifier: NCT01308580), in which patients previously treated with docetaxel will be randomized to receive cabazitaxel at either 20 or 25 mg/m^2, will address this in the post-docetaxel space. While it is tempting to speculate that the lower dose will result in less toxicity, based on exposure-response analysis that was performed on the 67 patients from TROPIC who had PK data collected, this dose reduction is expected to have minimal impact on the likelihood of experiencing grade 3 or higher neutropenia, with an absolute reduction of only 5 %, from 54 to 49 % [93].

This exposure-response analysis also explored whether higher drug exposure was associated with improved overall survival and longer time to progression, with an AUC cutoff of 907 ng•h/mL. Essentially there were too few patients included in this analysis to provide sufficient information, but patients with higher exposure had a numerically longer time to progression, but worse overall survival, likely confounded by four early deaths due to neutropenic complications.

Given the high toxicity burden seen with cabazitaxel, another question that arises is whether this treatment improves or maintains quality of life. This wasn't addressed in the TROPIC trial, where QOL data was not collected; however, data regarding pain, an important component of QOL was. Patients receiving cabazitaxel

showed similar pain response rates and time to pain progression as patients treated with mitoxantrone, which has previously been shown to improve pain control and QOL measures [7]. In the UK expanded access program, patients treated with cabazitaxel did have QOL measures formally assessed at alternate cycles using the EQ 5D-3L questionnaire and visual analogue scale. These assessments showed a slight trend towards improvement, with increasing benefit with the number of cycles received. While this improvement didn't meet statistical significance, it is difficult to drawn conclusions from this, as there were relatively few patients, at 112, and no comparator arm [101]. For instance, it may be possible that cabazitaxel maintains QOL, while patients treated with mitoxantrone may have experienced a relative deterioration. While this is just speculation, QOL will be formally assessed in the FIRSTANA and PROSELICA trials, which will give a valuable, prospective answer to this important question.

Another important consideration regarding cabazitaxel is whether this treatment is cost effective, as some jurisdictions, including the NHS in the UK for a brief period, have rejected funding for this drug, making it inaccessible to the majority of patients within those jurisdictions. The NHS decision was based on the modest OS benefit, high burden of toxicity, and lack of QOL data balanced against the high cost of this drug at 4435 GBP per cycle [125]. It is worth noting that cabazitaxel is significantly more expensive than abiraterone and enzalutamide, and similar in price to Radium-223 per cycle [126–128]. Furthermore, this doesn't take into account the additional costs of administering chemotherapy, such as nursing time, supportive medications, and management of complications. A cost effectiveness analysis was undertaken by the UK National Institute for Health and Clinical Excellence (NICE) and found that the cost per quality-adjusted life year (QALY) gained was 82,950 GBP, and thus concluded this was not a cost-effective use of resources and did not recommend cabazitaxel use [129].

Conclusions

Cabazitaxel represents an important advance in the field of castration resistant prostate cancer in that it was the first of a new wave of agents to show an improvement in overall survival in patients who had received prior docetaxel chemotherapy. Importantly, we have good prospective evidence that it is active in patients who are docetaxel-resistant or refractory, with retrospective evidence suggesting it is also active in patients who have received abiraterone and/or enzalutamide. While the toxicity burden is high, it is manageable through appropriate patient selection and supportive measures. Perhaps the biggest barrier facing the use of this agent is the financial cost associated with it, as some public health authorities are not supporting its use based on unfavourable cost-effectiveness analyses. Questions still remain over the use of this drug, such as the appropriate dose, treatment sequencing, use in combinations, and also how the positive results of docetaxel in the hormone-sensitive setting will impact its clinical utility.

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Sequencing Therapies in Metastatic Castration—Resistant Prostate Cancer

13

Michael T. Schweizer and Bruce Montgomery

Introduction

The approval of docetaxel in 2004 ended a nearly 60 year drought in which nothing was shown to prolong life for patients with metastatic castration-resistant prostate cancer (mCRPC), and ushered in a new era of treatment for mCRPC [1, 2]. Since then, six therapeutic agents (including docetaxel) have been approved for the treatment of metastatic castration-resistant prostate cancer (mCRPC)—all on the basis of Phase III data indicating a survival advantage with these drugs. These agents include chemotherapeutics (e.g. docetaxel and cabazitaxel), androgen receptor (AR) directed agents (e.g. abiraterone and enzalutamide), immunotherapeutics (i.e. sipuleucel-t) and radiopharmaceuticals (i.e. radium-223) (Fig. 13.1) [1–9].

Since docetaxel's approval in 2004, many subsequent approvals in the mCRPC therapeutic space have been predicated on prior docetaxel exposure (Fig. 13.2). For instance, the Phase III cabazitaxel study mandated that patients be post-docetaxel, and as such it remains only approved in docetaxel-treated patients [5]. Similarly, the approvals for abiraterone and enzalutamide were initially granted post-docetaxel, with approval for docetaxel-naïve patients only occurring after Phase III trials in that patient population were completed [3, 4, 8, 9]. While this regulatory framework was likely born out of the desire to select a patient population in which a survival benefit could be quickly demonstrated, it is not reflective of the current treatment

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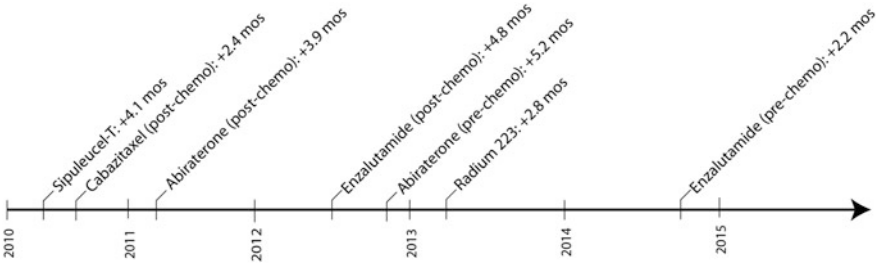

Fig. 13.1 Timeline of recent Food and Drug Administration (FDA) approval for mCRPC drugs. Median improvement in overall survival in months (mos) is provided

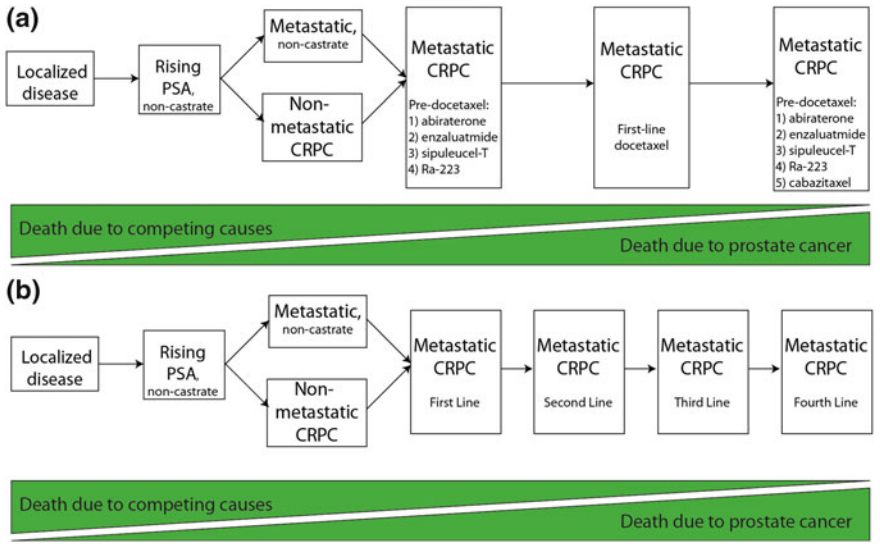

Fig. 13.2 Original prostate cancer clinical states model (**a**) and revised model (**b**). **a** Based on data from Ref. [11]; **b** Based on data from Ref. [10]

landscape in which many options exist for men with mCRPC. Furthermore, the Phase III studies testing these newer agents did not postulate biologic rationales for the specific therapeutic sequence being tested (i.e. pre- or post-docetaxel) or incorporate biomarkers to address the issue of optimal drug sequence, and there remains no historical or biologic basis for why AR targeting agents, such as abiraterone or enzalutamide, or cabazitaxel would *only* work if given after docetaxel. An acknowledgment of this point is reflected in the Prostate Cancer Working Group 3 (PCWG3) guidelines regarding the conduct of clinical trials in men with mCRPC. The PCWG3 guidelines provide a revised therapeutic framework, emphasizing the sequential use of approved agents, rather than selecting drugs based on a whether or not an individual has received prior docetaxel [10].

While some of these drugs have seemingly distinct mechanisms of action (e.g. radium-223 and sipuleucel-t), there is considerable mechanistic overlap between others (e.g. abiraterone and enzalutamide). As such, it is not surprising that evidence of cross-resistance between many of these therapies is being increasingly recognized. At the heart of this cross-resistance is the fact that many of our approved therapies rely on inhibiting the same target: the lineage-survival oncogene AR [12, 13]. In addition, increasing evidence indicates that prostate cancer evolves over the course of treatment, with more resistant subclones emerging, resulting in an inherently more difficult to treat disease [14–17].

Questions surrounding the optimal mCRPC treatment paradigm and how to sequence the six available mCRPC drugs remain. In this chapter, we will outline our current understanding of how to best utilize the drugs approved for men with mCRPC, and highlight some of the controversies surrounding when to use each of these agents.

Docetaxel

Taxanes (i.e. docetaxel and cabazitaxel) remain the only class of chemotherapeutics that result in improved overall survival compared to active controls for men with mCRPC [1, 2]. In recent years, several effective oral AR-directed therapies have been approved in the pre-docetaxel space, and as such practice patterns are beginning to shift towards delayed use of docetaxel in favor of these less toxic, and more easily administered agents [3, 8, 18].

Docetaxel still remains an important therapeutic option, however, and under certain circumstances may be preferred over the use of drugs like abiraterone or enzalutamide. For instance, docetaxel may be more appropriate if a patient requires rapid palliation or control of visceral metastases [19]. Docetaxel has been reported to achieve a pain response after 27 days and result in improved quality of life after 43 days [20]. To put this in context, the time to pain palliation with abiraterone has been reported to be 5.6 months [21]. While cross-trial comparisons are bias-prone, this difference is quite dramatic. It should also be noted that in both the abiraterone and enzalutamide pre-docetaxel Phase III studies, only patients who were asymptomatic or minimally symptomatic were permitted on study [3, 8]. Further complicating the issue is the recent data indicating that docetaxel results in substantial survival gains when used for hormone-sensitive metastatic prostate cancer, raising the question of whether the early use of docetaxel for men with mCRPC should be liberalized [22, 23].

For now the question regarding whether to sequence docetaxel before or after next-generation AR-directed therapies (i.e. abiraterone or enzalutamide) is not fully answered. Additional clinical trials are needed before any definitive conclusions regarding how to best utilize docetaxel in the context of effective and readily available oral agents remain.

Next Generation AR-Directed Therapies

For the majority of men with mCRPC, AR-signaling still constitutes an important driver of disease progression. Both tissue androgens and AR increase in tumor tissue as prostate cancer transitions to the castration-resistant state, and AR-regulated genes such as *PSA* are often expressed at high levels in men with mCRPC [24]. These observations led to renewed interest in targeting AR-signaling in mCRPC patients and subsequently prompted the development of drugs like abiraterone (an inhibitor of extragonadal androgen biosynthesis) and enzalutamide (a pure AR-antagonist) [3, 4, 8, 9, 25].

Both abiraterone and enzalutamide were initially developed in the post-docetaxel space, with their respective pivotal Phase III trials demonstrating a survival advantage compared to controls [4, 9]. Given that these studies were limited to a population that was exposed to prior docetaxel, initial drug approval was limited to patients that had already received docetaxel. Subsequent approvals in the pre-docetaxel space was only granted following publication of additional Phase III data showing a survival benefit when these drugs were used pre-docetaxel [3, 8, 26]. Consensus guidelines, such as the National Comprehensive Cancer Network (NCCN) Prostate Cancer Guidelines and the American Urological Association (AUA), recommend reserving docetaxel for patients with symptomatic, rapidly progressive or visceral disease [19, 27]. As such, practice patterns are likely shifting toward the earlier (i.e. pre-docetaxel) use of both abiraterone and enzalutamide [18].

In regard to the question of whether to use abiraterone or enzalutamide first, there are no clinical trials directly comparing these two agents to one another. Both drugs function similarly by inhibiting the ligand (e.g. testosterone, dihydrotestosterone [DHT]) AR interaction, and it is probably safe to assume that both agents have comparable efficacy when used as a first line treatment for mCRPC. The decision to choose one agent over the other may ultimately hinge on considerations independent of any anti-tumor effect. Abiraterone requires concurrent treatment with prednisone in order to blunt the mineralocorticoid side effects associated with its use [4, 8]. Therefore, in patients for whom steroids are contraindicated or undesirable (e.g. those with diabetes), enzalutamide may be preferred. It is also notable that in the pre-docetaxel enzalutamide Phase III trial patients with visceral disease were permitted; whereas, in the pre-docetaxel abiraterone Phase III trial these men were excluded [3, 8]. There is, however, no clear evidence that abiraterone is ineffective in treating visceral disease.

Radium-223

Radium-223 is a novel alpha emitting radiopharmaceutical that possesses intrinsic bone homing properties similar to that of other alkaline earth elements, such as calcium, and is approved for the treatment of symptomatic, bone-metastatic CRPC. Radium-223 is not expected to affect soft tissue metastases, and on this basis,

patients with visceral metastases or nodal metastases >3 cm in the short axis were excluded from the Phase III trial that led to radium-223's approval [7]. As such, it is only approved for mCRPC patients with metastatic disease predominantly affecting bone.

In contrast to abiraterone and enzalutamide, radium-223 was not tested in separate Phase III trials specifically designed for men that were pre- and post-docetaxel [7]. At baseline, fifty-seven percent of men enrolled to the radium-223 arm and placebo arm of the Phase III study had received prior docetaxel and the remainder were docetaxel naïve. As with abiraterone or enzalutamide, the indication for radium-223 is therefore agnostic of prior docetaxel treatment status.

In addition to location of metastatic disease, another consideration before initiating radium-223 is the fact that it did not clearly result in improved pain endpoints in its Phase III trial as assessed by validated pain assessment instruments. Given that a Phase II trial did document a palliative benefit, however, an ongoing observational study was designed to assess radium-223 effect on pain (clinicaltrials.gov: NCT02398526) [28]. Until the results of this study are reported, it should not be assumed that radium-223 would lead to a meaningful improvement in pain.

Cabazitaxel

Cabazitaxel is a newer taxane that was shown to result in prolonged survival compared to mitoxantrone when used after docetaxel [5]. As such, it is only approved for use following disease progression on a docetaxel-based regimen. While overall cabazitaxel resulted in a decreased risk of death compared to mitoxantrone, there was a higher risk of death within 30 days of receiving the last dose, likely reflecting the toxicity of this agent. This is in contrast to radium-223, which demonstrated comparable frequency of grade 3 and 4 adverse events compared to placebo. Whether cabazitaxel would prove to be as toxic in a less heavily pre-treated group of patients remains to be seen, and prospective studies assessing its effectiveness pre-docetaxel are currently underway (FIRSTANA trial [clinicaltrials.gov: NCT01308567] and TAXYNERGY trial [clinicaltrials.gov: NCT01718353]). Depending on the results of these trials, cabazitaxel use could potentially be approved for docetaxel-naïve patients, but for now its use in this patient population remains off-label.

Sipuleucel-T

Sipuleucel-T is an ex vivo autologous immunotherapy product and remains the only cancer vaccine shown to improve overall survival in Phase III testing [6, 29]. It is designed to produce an immune response toward the prostate antigen PAP. The Phase III study only included mCRPC patients with asymptomatic disease and an anticipated life expectancy of ≥6 months. The median survival of the placebo and

sipuleucel-t arms was long at 21.7 months and 25.8 months, respectively, likely reflecting the relatively good prognosis of the patients enrolled to the Phase III study [6]. Similar to the radium-223 Phase III trial, enrollment to the sipuleucel-t Phase III study was not limited based on prior docetaxel exposure, and ultimately 15.5 and 12.3 % of patients randomized to sipuleucel-t and placebo arms, respectively, were post-docetaxel. Interestingly no difference in disease progression was observed between study groups, and while this might call into question the overall survival benefit observed on this study, it should be noted that two additional randomized trials reported a survival advantage with sipuleucel-t [30, 31].

Based on the aforementioned, sipuleucel-t is not approved for patients with symptomatic disease, and given that only men with a life expectancy ≥6 months were included in the pivotal Phase III trial, it is not appropriate for patients with rapidly progressive disease or those expected to live <6 months. While it is technically approved pre- or post-docetaxel, in practice most men that have progressed on docetaxel are likely in need of a therapy that will result in objective tumor control.

Cross-Resistance

Surgical or medical castration (i.e. androgen deprivation therapy; ADT) as a treatment for prostate cancer was the first example of an effective targeted cancer therapy, and to this day inhibiting AR-signaling, primarily through disrupting ligand-AR interactions, remains the mainstay of treating advanced disease [25, 32]. Until recently, progression beyond frontline ADT was felt to represent an "androgen independent" or "hormone refractory" state; however, with the recognition that the AR transcriptional program is still operative in men that progress beyond ADT, the nomenclature has shifted such that these men are now referred to as having castration-resistant prostate cancer. As discussed above, many of the newer agents approved for the treatment of mCRPC function to inhibit AR-signaling, and not surprisingly, their long-term use can lead to the emergence of a drug resistant phenotype—manifested as diminished clinical activity when these drugs are used sequentially.

Abiraterone and Enzalutamide

Two of our most effective agents for treating mCRPC patients, abiraterone and enzalutamide, both function to inhibit AR ligands (e.g. DHT and testosterone) from binding the AR [25]. Abiraterone accomplishes this through inhibiting cytochrome P450-17 (CYP17), a key family of enzymes involved in gonadal, adrenal and intratumoral androgen synthesis [33–36]. The end result is testosterone levels that are significantly lower than those observed with ADT alone [37, 38]. Enzalutamide on the other hand is a pure AR-antagonist, which, unlike earlier anti-androgens

(e.g. bicalutamide, nilutamide and flutamide), is able to more completely antagonize the AR. In addition, it also prevents the nuclear translocation of the AR [39].

Given their similar mechanisms of action, it is not surprising that evidence of cross-resistance between abiraterone and enzalutamide has begun to emerge. A number of mechanisms of resistance have been described that may explain how continued AR-signaling occurs in spite of treatment with either abiraterone or enzalutamide. These include: upregulation of the AR; increased extragonadal androgen synthesis; the emergence of constitutively active AR splice variants (AR-Vs); AR point mutations; AR-signaling activation via alternative pathways (e.g. AKT/mTOR/Pi3 K, HER kinases); and activation of other nuclear hormone receptors such as the glucocorticoid receptor (GR) [14, 24, 40–49]. One or more of these mechanisms may provide a basis for why progression on one AR-directed agent may portend a poor response to the other drug when used second line.

Recently, evidence of clinical cross-resistance between abiraterone and enzalutamide has begun to emerge (Tables 13.1 and 13.2). While no randomized studies have evaluated if the sequence with which abiraterone and enzalutamide are given influences overall survival (i.e. abiraterone then enzalutamide vs. enzalutamide then abiraterone), several retrospective analyses have demonstrated decreased activity to the second line agent. For instance, abiraterone was reported to have a median progression free survival of 5.6 months in the Phase III trial testing it post-docetaxel [4]. When abiraterone is used to treat mCRPC patient post-docetaxel and enzalutamide, however, the median progression free survival has been reported at only 2.7 to 3.6 months [50, 51]. Likewise, when enzalutamide is given post-docetaxel and abiraterone, the median progression free survival has been reported to be 2.8 to 4.6 months, which is in contrast to the 8.3 month median progress free survival that was reported in the Phase III trial testing enzalutamide in patients that were post-docetaxel only [9, 52–56].

Upregulation of full length AR (AR-FL) and/or the presence of AR-Vs may be drivers of abiraterone and enzalutamide cross-resistance [14]. In a prospective study reported by Antonarakis et al., the presence of AR-V7 (the most prevalent AR-V) mRNA was determined by qRT-PCR on circulating tumor cells, and correlated with response (i.e. ≥50 % decline in PSA from baseline) to abiraterone (N = 31) or enzalutamide (N = 31) [14]. They reported that the presence of AR-V7 mRNA associated with a lack of PSA response to both abiraterone (0 % vs. 68 %, $P = 0.004$) and enzalutamide (0 % vs. 53 %, $P = 0.004$). In addition, time to PSA progression, clinical or radiographic progression and overall survival were all significantly shorter in men harboring an AR-V7. That study also found that high AR-FL transcript levels associated with a lack of response to abiraterone and enzalutamide. Interestingly, when compared to abiraterone and enzalutamide naïve patients, the prevalence of AR-V7 was higher in those that had been pre-treated with either abiraterone (55 % vs. 9 %) or enzalutamide (50 % vs. 15 %). In addition, pre-treatment with either agent led to high AR-FL transcript levels. It is plausible that the emergence of AR-Vs or the upregulation AR-FL following exposure to abiraterone or enzalutamide may at least partially explain the diminishing efficacy seen when these agents are used sequentially.

Table 13.1 Activity of abiraterone following treatment with enzalutamide and/or docetaxel

Reference	Agent	Clinical state	Study design	Sample size	Median overall survival (mos)	≥50 % PSA decline (%)	Median progression free survival (mos)	Objective response (%)
de Bono et al. [4]	Abiraterone	Post-docetaxel	Phase III	797	14.8	29	5.6	14
Loriot et al. [50]	Abiraterone	Post-enzalutamide and docetaxel	Retrospective review	38	7.2	8	2.7	8
Noonan et al. [51]	Abiraterone	Post-enzalutamide and docetaxel	Retrospective review	30	11.7	3	3.6	0

Note Sample size is only for the cohort treated with abiraterone

Table 13.2 Activity of enzalutamide following treatment with abiraterone and/or docetaxel

Reference	Agent	Clinical state	Study design	Sample size	Overall survival (mos)	≥50 % PSA decline (%)	Progression free survival (mos)	Objective response (%)
Scher et al. [9]	Enzalutamide	Post-docetaxel	Phase III	800	18.4	54	8.3	29
Schrader et al. [56]	Enzalutamide	Post-abiraterone and post-docetaxel	Retrospective review	35	7.1	28.6	4	2.9
Bianchini et al. [54]	Enzalutamide	Post-abiraterone and post-docetaxel	Retrospective review	39	–	12.8	2.8	4.3
Suzman et al. [71]	Enzalutamide	Post-abiraterone	Retrospective review	30	–	34	4.7	–
Badrising et al. [53]	Enzalutamide	Post-abiraterone and post-docetaxel	Retrospective review	61	7.4	21	2.8	–
Cheng et al. [55][b]	Enzalutamide	Post-abiraterone	Retrospective review	79	Not reached	18	4[a]	–
	Enzalutamide	Post-abiraterone and post-docetaxel	Retrospective review	165	–	28	2.8[a]	–
Azad et al. [52][b]	Enzalutamide	Post-abiraterone	Retrospective review	47	8.6	26	6.6	–
	Enzalutamide	Post-abiraterone and post-docetaxel	Retrospective review	68	10.6	22	4.6	–

Note Sample size is only for the cohort treated with enzalutamide
[a]Time to PSA progression is reported
[b]These studies report on overlapping cohorts

Table 13.3 Activity of docetaxel following treatment with abiraterone

Reference	Agent	Clinical state	Study design	Sample size	Overall survival (mos)	≥50 % PSA decline (%)	Progression free survival (mos)	Objective response (%)
Tannock et al. [2]	Docetaxel	Abiraterone naïve	Phase III	335*	18.9	45	7.7**	12
Mezynski et al. [68]	Docetaxel	Post-abiraterone	Retrospective review	35	12.5	26	4.6*	11
Schweizer et al. [18]	Docetaxel	Post-abiraterone	Retrospective review	24	–	38***	4.4***	–
		Abiraterone naïve		95	–	63***	7.6***	–
Azad et al. [67]	Docetaxel	Post-abiraterone	Retrospective review	37	–	32	–	–

Note Sample size is only for the cohort treated with docetaxel
*Only reporting on the cohort that received docetaxel 75 mg/m^2 every 21 days
**Time to PSA progression is reported
***$P < 0.05$

Taxanes and AR-Directed Agents

Somewhat surprisingly, evidence of cross-resistance between docetaxel and abiraterone has also recently begun to emerge (Table 13.3). Docetaxel likely exerts an anti-tumor effect through a variety of mechanisms independent of AR-signaling (e.g. impairing mitosis and inhibiting expression of the anti-apoptotic genes *Bcl-2* and *Bcl-x*) [57–61]. More recently pre-clinical studies have also demonstrated that docetaxel is also able to inhibit microtubule mediated AR trafficking into the nucleus, in theory preventing AR-signaling [62–66].

Similar to the aforementioned analyses that documented cross-resistance between abiraterone and enzalutamide, clinical evidence of cross-resistance between abiraterone and docetaxel has also begun to emerge. For instance, in the randomized Phase III trial that led to the approval of docetaxel, forty-five percent of patients had a ≥50 % decline in PSA (i.e. PSA response) from baseline [2]. This is in contrast to several retrospective analyses that have reported only 26–38 % of abiraterone pre-treated men achieving a PSA response to docetaxel [18, 67, 68]. In one retrospective analysis, the clinical outcomes on docetaxel for mCRPC patients who were either abiraterone naïve or post-abiraterone were compared [18]. In that study, the median time to PSA progression (6.7 vs. 4.1, $P = 0.002$), median clinical or radiographic progression free survival (7.6 vs. 4.4, $P = 0.003$) and PSA response (63 % vs. 38 %, $P = 0.02$) were all significantly better in the abiraterone naïve group. Of note, abiraterone treatment status remained a significant predictor of outcome when other clinically relevant covariates were controlled for through a multivariable model. It stands to reason that similar evidence of cross-resistance may also exist between enzalutamide and docetaxel, as well as between AR-directed agents and cabazitaxel; however, to our knowledge no such clinical data has been reported.

Conclusion

As the repertoire of approved agents for the treatment of mCRPC increases, so do the number of choices we face regarding which drug to select for any given patient. At this point, all approved therapies (except cabazitaxel) are indicated pre- and post-docetaxel. Recently presented consensus guidelines on the conduct of mCRPC clinical trials encourages investigators to avoid defining trial cohorts on the basis of chemotherapy treatment status—reflecting the new mCRPC therapeutic landscape [10]. To date, it remains unclear if an optimal sequence of mCRPC drugs exists; however, specific presenting clinical features may help steer the choice of when to use each agent.

Docetaxel is probably most appropriate if a rapid palliative response is needed (e.g. patients with rapidly progressive disease or visceral metastases) [2, 20]. On the other hand, abiraterone and enzalutamide both have excellent activity in patients with mCRPC, and in contrast to docetaxel, are generally better tolerated—often

making them more attractive choices for first-line mCRPC therapy, particularly for patients with significant comorbidities or impaired performance status [3, 4, 8, 9]. Evidence of cross-resistance between drugs that inhibit AR-signaling is becoming more apparent, and emerging clinical data indicates that adaptive changes in AR expression and/or emergence of constitutively active AR-Vs may drive resistance to chronic exposure to AR-directed drugs. Preliminary data indicates that docetaxel may be a better choice in the face of a resistant phenotype; however, whether there is an optimal sequence with which to use abiraterone, enzalutamide and docetaxel remains to be seen [69].

Sipuleucel-t, radium-223 and cabazitaxel have somewhat more restricted indications. Sipuleucel-t is only approved for asymptomatic patients, and as such is probably only useful in a small subset of mCRPC patients that present with slowly progressing disease [6]. It is important to note that sipuleucel-t will not prevent disease progression, so the appropriateness of delaying the initiation of drugs that can control disease (e.g. abiraterone, enzalutamide, docetaxel) must be considered. Radium-223 only targets bone metastases, and is not approved for patients with visceral disease or metastatic adenopathy >3 cm in size—limiting its usefulness in heavily pre-treated patients that are more likely to have soft tissue metastases [7]. Cabazitaxel is only approved following progression on docetaxel. In addition, it is relatively toxic and affords only a modest improvement in overall survival [5].

With so many agents to choose from, work towards developing predictive biomarkers should be prioritized. Ongoing efforts to characterize the molecular landscape of mCRPC through metastatic biopsy programs will likely play an important role in defining which group of patients stand to benefit from specific therapeutic agents. For instance, in a recent report by Robinson et al. [70] patients with mCRPC underwent a targeted biopsy followed by an integrative genomic assessment. In addition to documenting several previously described genomic aberrations (e.g. *AR*, ETS genes, *TP53* and *PTEN)*, this study also found a higher than expected frequency of biallelic loss of DNA damage repair pathway genes (e.g. *BRCA1*, *BRCA2* and *ATM*). This finding provides a rational basis for 'precision oncology' trials testing agents that either induce DNA damage or impair the DNA damage repair machinery (e.g. platinum chemotherapy, PARP inhibitor) in those with evidence of biallelic loss of DNA damage repair pathway genes.

Ultimately, prospective trials are needed to delineate the role each of the aforementioned drugs will play in treating mCRPC. Specific questions that need to be addressed include: (I) Are combinations of drugs better than their sequential use? (II) Is one sequence of drug use better than another? (III) Should every mCRPC patient receive every approved drug? (IV) Can biomarkers (e.g. AR expression level, AR-V status) lead to improved patient-drug selection? There has been a lot of progress made in the treatment of mCRPC over the past decade. Many questions remain, but with continued work we will learn how to most effectively use the plethora of agents now approved for mCRPC.

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Bone Preservation Strategies for Men on Androgen Deprivation Therapy

14

Charles C. Peyton and K.C. Balaji

Introduction—Bone Complications and Prostate Cancer

Prostate cancer was estimated to account for 26 % of new cancer diagnoses and 27,540 estimated deaths in the United States during 2015 [1]. Most deaths in men with prostate cancer are due to metastatic disease. The most common sites of hematogenous prostate cancer metastases are the axial skeleton, pelvis, long bones, and skull. Approximately 70–80 % men with advanced prostate cancer will develop bone metastases [2]. Furthermore, bone metastases has been reported in 90 % of men with metastatic prostate cancer at autopsy, far more common than visceral metastasis [3]. Other malignancies that commonly metastasize to bone (e.g. breast and lung) are commonly osteolytic; however, prostate cancer lesions are commonly osteoblastic. These metastases disrupt normal bone remodeling and induce architectural abnormalities leading to bone weakness [4]. The consequences of bone metastases in men with advanced prostate cancer represent an important disease process and economic burden [1].

Increased osteoblastic activity depletes serum calcium causing hypocalcemia and subsequent secondary hyperparathyroidism is often observed [5, 6]. Secondary anemia due to bone marrow suppression and cancer therapies is also common

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among men with metastatic prostate cancer and correlates with prognosis [7–10]. Bone metastases cause significant local pain symptoms and serious skeletal complications such as pathologic fracture, spinal cord compression and possibly require radiation or surgery. These complications can be further exacerbated in patients undergoing androgen deprivation therapy (ADT) for prostate cancer. As the U.S. population ages osteoporosis-related fractures have become an important national health concern and economic challenges predicted to increase 50 % by 2025 [11, 12]. Glucocorticoid excess, alcohol abuse and hypogonadism are the major causes of osteoporosis in ageing men [13]. Androgen deprivation therapy (ADT) is a well-understood pillar of systemic therapy for prostate cancer and the intended consequence is hypogonadism. Since the adoption of prostate specific antigen (PSA) testing, the prevalence of ADT among older men is rising [14]. The majority of men with metastatic prostate cancer and a substantial portion of men with localized recurrence and/or receiving concomitant radiation will be placed on ADT.

Several studies have clearly demonstrated the relationship between ADT induced hypogonadism and increased risk of skeletal fractures, particularly after long-term use [15, 16]. Furthermore, fractures in men with prostate cancer are particularly concerning due to their association with increased mortality [17]. Consequently, understanding and managing the skeletal consequences of advanced prostate cancer is an important aspect of providing comprehensive care. The objective of this chapter is to review the pathophysiology, current therapeutic interventions and clinical trial data for the prevention and treatment of bone complications in patients undergoing ADT for prostate cancer.

Bone Physiology

Normal bone physiology is a complex process of homeostasis intended to remodel bone for strength and mineral compositional stability. Osteoclast and osteoblast differentiation and activation are an important step in the complexity of bone remodeling [18]. An imbalance in the activation of monocyte-macrophage precursor cells to differentiate into osteoclasts results in secretion of proteases designed to dissolve bone matrix. Release of the bone marrow extracellular matrix growth factors enhances the microenvironment growth potential [19]. Activation of precursor monocytes is dependent on macrophage colony stimulating factor and receptor activator of nuclear factor–κB (RANK) and its ligand (RANKL), a member of the tumor necrosis factor (TNF) superfamily. Pro-resorptive factors such as 1,25-dihydroxyvitamin D, parathyroid hormone (PTH), parathyroid hormone related protein (PTHrP) and prostaglandins induces the release of RANKL by osteoblasts, stromal cells and activated T cell lymphocytes. RANKL binds to RANK on osteoclast precursor cells leading to differentiation, activation and survival [18].

Activated osteoclasts undergo structural changes to favor adherence to bone matrix and they begin secreting lytic enzymes to degraded bone and release calcium. The counter-balance to osteoclast activation is the secretion of osteoprotegerin (OPG) from osteoblasts and stromal cells. OPG competitively inhibits RANK

signaling by binding competitively binding RANKL [18, 19]. Anabolic signals for OPG secretion include estrogens, calcitonin, transforming growth factor-β (TGF-β), platelet derived growth factor (PDGF) and bone morphogenetic proteins (BMP) [18, 20]. Following osteoclast inhibition, mature osteoblasts lay down a connective tissue matrix that is mineralized to become bone. Basic science studies have confirmed that OPG overexpression leads to osteopetrosis and deletion of OPG causes osteopenia [21, 22]. The tight regulation of osteoclast and osteoblast activation balances bone homeostasis.

Mechanisms of Prostate Cancer Bone Metastasis

Several unique features explain the propensity for cancer cells to target bone. The vertebral-venous plexus extending from the pelvis through the paravertebral veins may explain the tendency of prostate cancer to favor the axial skeletal [2]. Bone is a rich repository for growth factors such as TGF-β, fibroblast growth factors, PDGF, insulin-like growth factor and BMP that are released during bone resorption (osteoclast activity) thereby favoring tumor cell implantation and growth [19].

Prostate cancer bone metastases are predominantly osteoblastic [23]. Bone weakness and enhanced osteoblastic activity of prostate cancer seems to be counterintuitive. However, rapidly constructed, poorly woven bone is structurally weak. Osteoclast activity is increased and markers of bone resorption such as elevated alkaline phosphatase are observed with osteoblastic metastases. Furthermore, PTH-related peptide (PTHrP) is often secreted by metastatic cells and activates osteoclasts by inducing RANKL [18, 19]. Concurrent osteoblastic activity causes hypocalcemia thereby stimulating PTH release, leading to secondary hyperparathyroidism and further osteoclast activation. The cycle propagates osteoblastic activity via liberation of growth factors from bone matrix and tumor cell proliferation [4, 18, 19]. However, it is unclear if bone destruction precedes osteoblastic metastasis or vice versa [19]. The interactions between tumor cells, osteoblasts, osteoclasts and the bone microenvironment are illustrated in Fig. 14.1.

Effect of Androgen Deprivation Therapy on Bone Mineral Density and Osteoporosis

Osteoporosis is defined as a BMD less than or equal to a score 2.5 standard deviations below that of a normal young male [24]. However, most fractures in elderly men do not usually occur in the osteoporotic range [25]. Thus the World Health Organization (WHO) endorsed an updated method of fracture risk assessment called the Fracture Risk Assessment Tool (FRAX) based on clinical risk factors for fracture and femoral neck BMD. Application of this risk calculator revealed that almost a third of U.S. men over 65 would qualify for treatment of osteoporosis [26]. Saylor and colleagues applied the FRAX calculator to 363 patients treated with ADT for prostate cancer. Over 50 % of men would have been

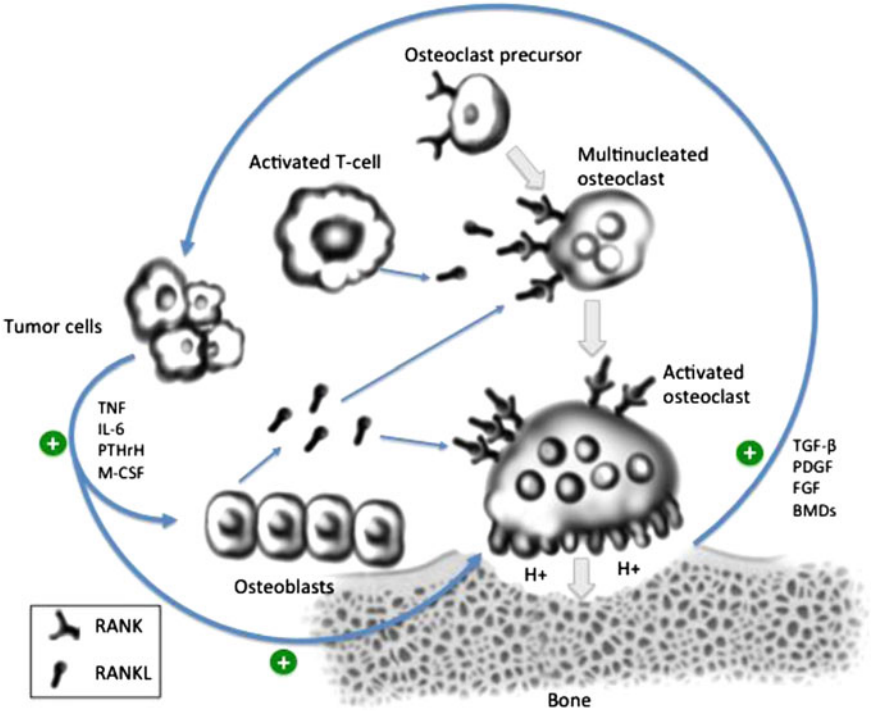

Fig. 14.1 Illustration of the relationship between tumor cells, osteoblasts, osteoclasts, bone microenvironment and the RANK-signaling pathway. Pro-resorptive factors such as $1,25(OH)_2$ vitamin D_3, parathyroid hormone (PTH), prolactin, corticosteroids, tumor necrosis factor (TNF), interlukin-1 (IL-1) and IL-6 trigger normal hormonal control of osteoclast activation through the RANK pathway (not pictured). However, metastatic tumor cells secrete cytokines and factors such as TNF, IL-6, parathyroid related hormone (PTHrH) and macrophage colony-stimulating factor (M-CSF) that activates osteoblastic activity, RANKL production, and osteoclast activation. Activated T cells also release RANKL. Binding of RANKL to RANK on osteoclast precursors induces differentiation and activation of mature osteoclasts. Bone resorption releases a microenvironment of growth factors such as transforming growth factor β (TGF- β), platelet-derived growth factor (PDGF), fibroblast growth factors (FGF), and bone morphogenetic proteins (BMPs) which promote tumor cell proliferation and further stimulation of cycle [18, 19]

recommended for fracture prevention therapy [27]. Certain studies have demonstrated skeletal related events (SREs) occurring in up to 40–50 % of men with castrate resistant metastatic prostate [28, 29].

National retrospective Medicare claims data suggests a 45 % incidence of ADT use for prostate cancer patients within the first year of diagnosis between 2000 and 2002 [30]. Regardless of surgical or hormonally induced hypogonadism, ADT causes rapid loss of bone mineral density (BMD) within the first year of therapy, and declining BMD is related to skeletal related events (SRE). BMD is traditionally measured via dual-energy absorptiometry through a bone densitometer. Reported

rates of BMD loss with in the first year of ADT range from 2.5 to 7.6 % with greatest loss at the spine, and average BMD losses decreased to 1.4 to 2.6 % in the following years [31–33]. Furthermore, ADT heavily influences systemic metabolic changes leading to obesity, insulin insensitivity and lipid profile changes, which may further stress changes in BMD [34].

The sex steroids influence on bone health is essential in regulating bone remodeling [35]. Estrogen has been established as a primary influence over bone remodeling in women and men [35]. Specifically, estrogens decreased osteocyte and osteoblast apoptosis, inhibit osteoclast activation directly or via osteoblast and T-cell influence to decrease RANKL levels. This ultimately leads to decreased bone remodeling and promotes stability [36]. Men with lower estradiol levels are at higher risk of fracture [37]. Peripheral aromatization of testosterone results in estradiol formation, thus hypogonadal men are at high risk for low estrogen levels [38]. Smith et al. [39] report an approximately 75 % reduction in estradiol levels in 48 men with recurrent prostate cancer and no metastatic disease that received leuprolide after 24 weeks. Low estrogen levels are an established connection between hypogonadism, decreased BMD and increased risk of fracture [15, 16, 40].

Regardless of metastases, patient on ADT are at higher risk of fracture. In 2005 Shahinian et al. perform a large retrospective review of 50,613 men using the Surveillance, Epidemiology and End Results (SEER) database with a diagnosis of prostate cancer from 1992 to 1997. Almost 20 % of those men who received ADT suffered a fracture as compared to only 12.6 % of men who did not received ADT ($P < 0.001$). The relative risk of fracture was 1.54 (95 % CI 1.42–1.68) for those having received 12 months or greater of ADT. The risk of fracture increased with duration of therapy regardless of metastatic disease [15]. Smith et al. [41] further confirmed this point in another large Medicare claims study of over 11,000 men using gonadotropin-releasing hormone (GnRH) agonist therapy.

Mechanisms of Bone Preservation Therapies

Bone Targeted Strategies

Bisphosphonates

Inorganic pyrophosphates are naturally occurring components of bone matrix with high affinity for calcium crystals and functions to impair calcium dissolution. However, exogenous pyrophosphates are quickly metabolized. Bisphosphonates are stable analogs of pyrophosphates first developed in the 19th century by substituting a carbon for oxygen between two phosphate groups (PO_3) [42]. Bisphosphonates tightly bind hydroxyapatite crystals of exposed bone thus decreasing the calcium binding capacity of osteoclasts. Osteoclast endocytosis of bisphosphonates causes direct inhibition of osteoclast activity by binding and inactivating farnesyl pyrophosphate synthases (FPPS), an important enzyme in cholesterol biosynthesis, which plays a role in

intracellular GTPase molecular signaling [43]. This promotes changes in the cytoskeleton of osteoclasts and induces loss of their ruffle-border and intravesicular trafficking which leads to inactivation and apoptosis. Non-nitrogen containing bisphosphonates combine with osteoclast adenosine-tri-phosphate (ATP) formation and cause apoptosis [44]. Non-nitrogen containing, first-generation bisphosphonates include etidronate, clodronate and tiludronate. Nitrogen containing side chains (usually an amino group) improve the potency of the drug and its composition determines the mechanism of action. Nitrogen containing, second generation bisphosphonates include pamidronate and alendronate. Third generation, highly potent, nitrogen rich bisphosphonates are neridronate, risedronate, ibandronate, zoledronate [44]. These potent bisphosphontate contain a secondary or tertiary amino group what are 100–1000 times more potent than first or second-generation bisphosphonates [4]. Bisphosphonates, in general, have very low biologic availability through the gastrointestinal tract, and intravenous administration is necessary for several of the drugs. [45]

Adverse side effects of bisphosphonates include nephrotoxicity, hypocalcaemia, osteonecrosis of the jaw (ONJ) and acute phase reactions. More potent intravenous bisphosphonates such as pamidronate and zoledronate have greater potential for adverse reactions and occasional dose modifications are required for renal impairment [46]. High dose pamidronate has been associated with risk of nephrotic syndrome, specifically focal segmental glomerular sclerosis (FSGS). Conversely, zoledronate has been associated with damage to the tubules causing acute tubular necrosis (ATN) [46]. Both complications are related to high dose administration and quite rare in general. Discontinuation of the bisphosphonate leads to improvement in renal function.

Osteonecrosis of the jaw (ONJ) is a rare but severe complication of bisphosphonates. High dose bisphosphonate therapy for oncology patients appears results in an estimated incidence of 1–12 % at 36 months after exposure. The rates of ONJ for osteoporosis related (non-oncology) therapy is minimal, <1 case per 100,000 person-years exposure is the quoted incidence [47]. Risk factors for ONJ include periodontal disease, trauma, head and neck radiation therapy, malignancy, chemotherapy, tooth extraction, glucocorticoids and high-dose IV bisphosphonates [47]. Thus, it is recommended that patients initiating IV bisphosphonate therapy undergo a routine clinic dental exam with panoramic radiographs. There is no evidence to support withholding bisphosphonate therapy in cancer patients at risk for SRE based on the potential risk of ONJ [48]. Currently with Southwest Oncology Group (SWOG) is conducting a large prospective trial tracking the incidence, risk factors and results of ONJ aiming to enroll 7200 patients [49]. Finally, almost one third of patients receiving intravenous zolendronic acid are at risk for an acute phase reaction. Symptoms include pyrexia, myalgia, flu-like symptoms, weakness, arthralgia and headache. The symptoms are transient and usually resolve within 72 h commonly with the assistance of anti-inflammatory medications. After having experience on episode of acute phase reaction, the likelihood of subsequent reactions are much lower [44, 50].

RANKL Inhibitors

Denosumab (Amgen Inc. Thousand Oaks, CA) is a human monoclonal IgG_2 antibody with high binding capacity for RANKL. The antibody mimics osteoprotegrin (OPG), the natural decoy receptor for RANKL and therefore decreases differentiation and activation of osteoclasts by preventing RANK binding [51]. The inhibitory effects of denosumab are longer lasting than bisphosphonates because it has a maximum circulatory half-life of 32 days, which enhances its long-term effectiveness [4, 51, 52]. On the other hand, bisphosphonates disappear very rapidly to the bone, which can limit its effectiveness in bones not undergoing active turnover [45]. The original phase I studies of denosumab evaluated response in postmenopausal women with breast cancer or multiple myeloma. After a single dose of denosumab, bone antiresorptive effects (decreased urinary and serum N-telopeptide levels) were noted within 24 h and sustained for 84 days [53]. Overall, the early phase I studies suggested that denosumab was well tolerated and achieved rapid and sustained osteoclast suppression [51, 53].

Chronic kidney disease is an additional risk factor for bone loss and should be considered when planning bone preservation strategies. Zoledronic acid is contraindicated in patients with GFR <35 mL/min/1.73 m^2 for osteoporosis, but used with dose adjustment for patients with skeletal metastases. An added benefit to denosumab is that it can be used at its regular dose in patients with impaired renal function. However, these patients are more prone to hypocalcemia; therefore, supplementation with calcium and vitamin D is recommended in these patients [54].

Osteoprotegerin

Osteoprotegerin (OPG) is an endogenous RANKL inhibitor. Recombinant OPG has been synthesized and attached to immunoglobulin heavy chains. Bekker et al. tested recombinant OPG effects with a single dose in postmenopausal women. The injection was well tolerated and rapidly reduced bone turnover measured by urinary N-telopeptide, deoxypyridinoline and serum bone-specific alkaline phosphatase. Urine markers were decreased within 12 h and bone-specific alkaline phosphatase decreased within weeks [55]. Another phase I study of recombinant OPG called AMGN-0007 also confirmed to this drug to be a well tolerated, rapid and sustained suppressor of bone resorption in patients with multiple myeloma and breast cancer [56].

The toxicities associated with recombinant OPG are fairly mild in the two human studies. The most common reported adverse events were fatigue, myalgia, bone pain and upper respiratory infection [56]. Although these results were suggestive that recombinant OPG can reduce bone resorption marker levels and possibly be as effective as pamidornate in reducing bone metabolism, it is unclear and unproven if this information will translate into a decrease in skeletal related events [55, 56]. However, recombinant OPG has not become favorable for several reasons. One possibility is that recombinant OPG could induce production of anti-recombinant OPG antibodies and cross-react with native OPG, thereby limiting its endogenous function. Also, the binding of OPG to endogenous TNF-related apoptosis inducing

ligand (TRAIL) could block normal host defenses against irregular cell growth [4]. Lastly, RANKL inhibitors seem to have a longer period of activity and a greater decrease in bone turnover [4, 55]. Given these limitations, denosumab has been the frontrunner for bone preservation.

Radiopharmaceuticals

Radiopharmaceuticals target the metastatic bony microenvironment and have become another option in bone preservation strategies. The compounds are structurally similar to calcium and therefore localize to the sites of osteoblastic activity. The surrounding abnormal cells are destroyed by local radiation delivery of β or α particle emission. The ideal radiopharmaceuticals are released quickly upon bony uptake and have an adequate half-life to provide therapeutic effect but limit myelosuppression [57]. Strontium-89 and samarium-153 are pure β-emitters that can be used in patients with multifocal, symptomatic bony metastatic prostate cancer for palliative pain control [57]. Given these findings and the limited benefit, difficulty of administration and myelosuppression, Strontinum-89 and samarium-153 have fallen out of favor. Radium-223 is the first agent to show an overall survival benefit for men with castration resistant metastatic prostate cancer [58] and delay in median time to first SRE [59].

Strontium-89 (Sr-89)

Sr-89 was first approved by the Food and Drug Administration (FDA) in 1993 for the treatment of painful bone metastases [60]. The agent is a pure β-emitter at a maximum energy of 1.47 MeV, long half-life of 50.5 days and soft-tissue penetration of 2.4 mm [57]. Several randomized control trials confirmed moderate improvement in pain control for patients with metastatic prostate cancer treated with Sr-89 compared to localized external beam radiation [61] and in the adjuvant setting [62]. However, other randomized studies reported no difference in pain response vs. placebo [63]. A systematic review of the efficacy of Sr-89 published in 2005 suggests a mean efficacy of 32 % for complete pain response and 44 % for partial pain response. No differences were noted in overall survivial [64]. The toxicities associated with Sr-89 are usually tolerable. Leukopenia and thrombocytopenia can be common with an incidence of 20–80 %. Serious hematologic adverse events are rare; however, routine hematologic monitoring is necessary [57]. The inconsistent results and advent of new radiopharmaceuticals has limited the use of Sr-89 in recent years.

Samarium-153 (Sm-153)

Initial studies of Sm-153 quickly illustrated its preferential affinity for hydroxyapatite, which is in higher concentrations at metastatic bony lesions [65]. Concentrating the dose at metastatic lesions allows for less systemic toxicity, lower energy delivery (β-emitter at 0.22 MeV) and rapid serum clearance. Only 4–34 % of the injected dose remains in serum after 1 h [57, 65]. Kidneys are the main source elimination and there is no change in excretion with dose variation [57]. Two phase-III studies have demonstrated the safety and efficacy of Sm-153 to provide

improved pain relief in metastatic prostate cancer but without any improvement in overall survival [66, 67]. Sartor et al. randomized 152 patients with bone metastatic castration resistant prostate cancer (mCRPC) to radioactive or nonradioactive Sm-153 and measured validated patient derived visual analog scales and pain descriptors. Patients reported significant improvements in 38 % of the treatment group versus 18 % of the placebo group. Myelosuppression was reported in 3–5 % and no overall survival advantage was noted [66].

Radium-223

In contrast to Sr-89 and SM-153, Radium-223 (Ra-223) chloride is a α-particle emitter. The compound mimics calcium for bone targeting and the α-particle emission provides dense ionizing radiation with a narrow range of <100 μm and high-energy transmission. The focused α-particles corresponds to about 2–10 cells in diameter, which minimizes the myelotoxicity of the surrounding healthy bone [68]. The α-particles cause DNA double strand breaks leading to cessation of the cell growth cycle and cell death [57]. Other advantages of Ra-223 include its bone-seeking properties, rapid gastrointestinal clearance of particles not taken up by bone and suitable half-life of 11.4 days [68]. Initial phase-I clinical trials subjected 25 breast and prostate cancer patient to a dose-escalation study design. Dose-limiting side effects were defined. Less than 1 % of the initial Ra-223 dose remained in the serum at 24 h, dose-limiting hematological toxicity was not observed and encouraging pain relief results were reported [68]. A phase-II double-blind placebo-controlled trial randomized 64 CRPC patients to Ra-223 versus placebo. Ra-223 induced significant reductions in all bone biomarkers and delayed time in PSA progression. The study did not demonstrated delay in SRE or statistically significant improved overall survival; however, these were not primary end points [69]. Hematological toxicities with Ra-223 were minimal. Phase III study results are discussed the in clinical trial section of this chapter.

Disease Targeted Strategies

Lifestyle Modifications

Recommending lifestyle changes can be a first step in managing metastatic prostate cancer patients. Not surprisingly, tobacco use, excessive alcohol consumption, and caffeine use are associated with increased risk for osteoporosis and fracture. Patients should be counseled on smoking cessation and moderations of alcohol and/or caffeine consumption [70]. Physical activity and weight bearing exercises have been associated with decreased risk of hip fractures and preservation of bone density. Adults are recommended to participate in at least 30 min of moderate physical activity daily including a mix of weight-bearing and balanced training exercises [71]. Patients should be counseled on fall prevention and assessed for changes in vision, hearing, neurologic changes or medications changes that could put them at increased risk for fall [70].

Calcium and Vitamin D

A key biologic function of vitamin D and calcium is to maintain bone mineralization and the National Osteoporosis Foundation (NOF) recommends calcium and vitamin D supplementation for adults over 50 at risk for osteoporosis (800–1000 IU vitamin D and 1200 mg daily calcium) [70]. A 2011 meta-analysis for the U.S. Preventative Task Force (USPTF) analyzed 19 randomized control trials (RCTs) and 28 observational studies regarding vitamin D supplementation with or without calcium and fracture risk. The pooled relative risk was decreased for older adults (RR 0.88; 95 % CI 0.78–0.99). However, most trial participants were older post-menopausal women and when stratified for institutionalized vs. community dwelling adults, only institutionalized adults showed any benefit [72]. Therefore it is difficult to draw broad conclusions regarding men with prostate cancer from this meta-analysis.

The NCCN Prostate Cancer Guidelines (available at www.nccn.org) suggest a baseline BMD exam and vitamin D deficiency-screening test. They also suggest supplementation with calcium (500 mg) and vitamin D (400 IU) for men being treated with ADT [73]. For those patients with <20 ng/mL 25-OH-D they should be replaced aggressively with 50,000 IU of vitamin D weekly for 8 weeks [74]. However, there are no published randomized control trials comparing vitamin D and calcium supplementation to placebo [75]. The recommendations are largely drawn from large studies of post-menopausal women demonstrating a decreased risk of fractures in those women adequately supplemented [72]. Furthermore, several clinical trials have shown that men undergoing ADT continue to lose BMD despite receiving the commonly recommended dose of calcium and vitamin D [75]. While supplementing men with prostate cancer on ADT with calcium and vitamin D makes intuitive sense, caution should be exercised. Excessive calcium intake (>1500 mg/day) is associated with increased risk of advanced or fatal prostate cancer based on large epidemiological studies [76, 77].

Selective Estrogen Receptor Modulators

Estradiol is critical to bone formation and resorption in men [36]. Peripheral aromatase converts testosterone to estradiol. Therefore, ADT induced hypogonadism in men will cause secondary decrease in estrogen levels and unintended bone weakening consequences. Furthermore, ADT likely causes serum elevation of triglycerides and cholesterol due to estrogen receptor mediated changes in anabolic hepatic receptor expression [78]. Selective estrogen receptor modulators (SERMs) exhibit full or partial estrogen agonist activity on bones and serum lipid levels, yet also exhibit estrogen antagonist activity on the breast [79, 80]. The effects of SERMs in women are well understood and are often used to attenuate the symptomatic, osteoporotic and cardiovascular effects of menopause in women. However, the effects of SERMs in men with prostate cancer undergoing ADT are less well defined. Two studies have provided randomized control trial data in support of bone preservation in men with prostate cancer on ADT [78, 81].

The major concerning toxicity associated with SERMs is increased risk of deep venous thromboembolism (VET), deep vein thrombosis (DVT) and pulmonary embolism (PE). This is certainly a risk that must be considered when initiating therapy. Treatment with toremifene in the setting of ADT for prostate cancer was associated with increased risk of VTE by 2.6 % versus placebo at 1.1 % [78]. SERM therapy in women with breast cancer has a risk of 1–3 % for PE, VTE or DVT [80].

Novel Anti-androgens

Abiraterone and enzalutamide are new anti-androgen agents that systemically control the metastatic disease, improve survival and attenuate the impact of skeletal metastasis. Although these agents do not specifically target bone, their effects decrease the rate of SREs. Abiraterone is an up-stream, selective inhibitor of the CYP17 enzyme thereby blocking androgen biosynthesis in the adrenal, testes and prostate. Despite additional androgen blockage, abiraterone after chemotherapy has also demonstrated delay in SRE by nearly 5 months (25 vs. 20.3 months; $p = 0.0001$) and improved pain control [82]. Almost 50 % of these patients were receiving bisphosphonate therapy at the time of abiraterone treatment, making the results just that much more impressive. This suggests that the addition of abiraterone to bisphosphonate therapy may be additive bone preservation therapy [82].

Enzalutamide is an androgen receptor (AR) antagonist that competitively targets the receptor with a fivefold higher binding affinity than bicalutamide. It also inhibits AR translocation to the nucleus and DNA binding [83]. Clinical trial data suggest enzalutamide prolongs time to first SRE (16.7 vs. 13.3 months respectively, HR 0.69, 95 % CI 0.57–0.84; $p = 0.0001$), accounting for a 31 % risk reduction in SRE [84]. Systemic non-bone directed therapy has proven to be beneficial in terms of overall survival, preventing SREs and treating bone pain.

Molecular Targets

Cabozantinib

Abnormal signaling in receptor tyrosine kinase (RTK) pathways is often present in cancer cells [85]. The RTK cMET proto-oncogene is frequently over expressed in prostate cancer [86]. Cabozantinib (XL184) is a multi-kinase inhibitor targeting VEGF receptors and cMET kinase signaling. Preclinical studies suggest that the MET pathway has effects on BMP-2 and activated osteoblasts [87]. For men with metastatic CRPC, phase II clinical trial data demonstrated 68 % improvement in radiographic bone disease and resolution in 12 %. Pain control improved by 67 % [88]. The drug was generally well tolerated and adverse effects included fatigue, hypertension and hand-foot syndrome. However, results of the phase III trial (COMET-1; NCT01605227) comparing a lower dose (60 mg/day) cabozantinib versus prednisone in men with mCRPC previously treated with docetaxel and abiraterone or enzalutmide did not improve overall survival [89]. A second trial (COMET-2; NCT01522443) comparing the effect on pain and bone scans with

cabozantanib versus mitoxantrone plus prednisone was canceled following the results of COMET-1.

Src Kinase Inhibition

Src is a nonreceptor tyrosine kinase that plays an important role in metastatic lesions within the bone microenvironment. The molecular switch activates osteoclasts and promotes their survival [90, 91]. Src is up regulated in CRPC [92]. Inhibitors of Src such as dasatinib prevent prostate cancer cell adhesion and invasion at skeletal lesions in pre-clinical studies [93]. A phase II trial combining dasatinib with docetaxel in chemotherapy-naïve CRPC patients demonstrated moderate improvement in bone scans and bone-specific ALP [94]. However, phase III READY trial randomized 1522 mCRPC patients to docetaxel plus dasatinib vs. docetaxel plus placebo and failed to demonstrate significant difference in median overall survival [95]. There was a suggestion of delayed time to first SRE in the dasatinib group (median time not reached vs. 31.1 months in placebo), but these results were considered coincidental given the negative primary endpoint [95].

Phase 3 Clinical Trials Summary

Bone Targeted Therapies for Bone Metastatic Prostate Cancer—Table 14.1

Bisphophonates

There are several notable phase 3 clinical trials evaluating the efficacy of bisphosphonates in patients with metastatic prostate cancer since the millennium. Both castration resistant and sensitive populations have been evaluated. Intravenous bisphosphonate therapy is considered to be standard of care for bony metastatic disease by many oncologists. However, development of newer agents such as denosumab may be changing that paradigm.

Zolendronic Acid—Metastatic CRPC

In the *Zometa 039* study 643 patients with metastatic CRPC were randomized to double-blind treatment regiment of intravenous zolendronic acid 4 mg (214 patients), 8 mg (221 patients) or placebo (208) every three weeks for 15 months [29, 96]. All men were continued on ADT (pharmacologic or surgical) throughout the study. Initiation of chemotherapy was at the discretion of the provider. The primary end point was time to first SRE defined as pathologic fracture, spinal cord compression, require surgical and/or radiation therapy to bone, and changes to include chemotherapy to treat bone pain. Secondary endpoints included pain and disease progression. Patients were excluded if they were on cytotoxic chemotherapy at time of enrollment, had received radiation therapy within 3 months, serum creatinine >3 mg/dL or had hypo- or hypercalcemia. All patients were on calcium and vitamin D supplementation.

Table 14.1 Randomized control trials of bone targeted therapies for bone metastatic prostate cancer

	Trial	*n*	Study population	Arms	Primary endpoint	Results	Comments
Bisphosphonates	Zometa 039 [29, 96]	643	mCRPC	Zoledronic acid 4 mg versus placebo every 3 weeks	Prevalence of SRE at 15 months	33 % versus 44 % (p = 0.021) Time to SRE 488 versus 321 days (p = 0.009)	8 mg arm dose reduced to 4 mg after noted nephrotoxicity
	CLAGB 90202 [97]	645	Castrate sensitive metastatic PC initiated on ADT within 6 months	Zoledronic acid 4 mg versus placebo every 4 weeks	Time to first SRE	Median time to SRE 31.9 versus 29.8 (p = 0.39)	Study terminated prior to full accrual Overall survival between groups similar
	CGP-32 and INT 05 [98]	350	mCRPC	90 mg pamidronate versus placebo every 3 weeks for 27 weeks	Pain, analgesic use, SRE	No difference	Oral bisphosphonates are not potent enough
	NCIC CTG PR.6 [99]	209	mCRPC	Mitoxantrone + prednisone ± 1500 mg clodronate every 3 weeks until progression versus placebo	Pain index scores, analgesic use, quality of life	46 % versus 39 % palliative response (p = 0.54)	Clodronate not useful for palliation
	MRC PR05	311	Castration sensitive, metastatic PC on ADT	2080 mg clodronate daily versus placebo for 3 years	Symptomatic progression or death	Trend to prolonged progression-free survival (p = 0.066)	Improved long-term OS with for clodronate (5-years 30 % vs. 21 % alive; HR: 0.77, p = 0.032)
Denosumab	Denosumab 103 [102]	1901	mCRPC	120 mg denosumab subcutaneous versus 4 mg zoledronic acid every 4 weeks	Non-inferiority, time to SRE	Denosumab was non-inferior, median time to SRE 20.7 months versus 17.1 months (p = 0.0002)	Secondary endpoint: denosumab was superior to zoledronic acid; no difference in OS or adverse event rate

(continued)

Table 14.1 (continued)

	Trial	*n*	Study population	Arms	Primary endpoint	Results	Comments
Radium-223	ALSYMPCA [58, 59]	921	mCRPC with symptomatic bone metastases and no visceral metstases	6 treatments Radium-223 50 kBq/kg versus placebo every 4 weeks	Overall survival	Median overall survival improved by 2.8 months with Radium-223 (HR: 0.65, $p = 0.0019$)	Study terminated early in recognition of benefit; secondary endpoint: fewer SRE and longer time to SRE in treatment arm (13.8 vs. 8.4 months, $p = 0.0005$)

ADT Androgen depravation therapy, *HR* hazard ratio, *mCRCP* metastatic, castration-resistant prostate cancer, *OS* overall survival, *PC* prostate cancer, *SRE* skeletal related event

At 15 months fewer men in the treatment group experienced SRE than the placebo group (33 % vs. 44 %, $p = 0.021$) [96]. The long-term follow up revealed significantly longer median time to SRE in the treatment group (488 vs. 321 days, $p = 0.009$) [29]. Pain and analgesic scores were significantly higher in those patients who received placebo. Although the study was not powered to evaluate survival, the median overall survival of patients in the zoledronic acid 4 mg group was numerically larger but not significant (546 vs. 464 days, $p = 0.91$). Zoledronic acid was continued even if patients had SRE and multiple-event, ongoing analysis demonstrated a 36 % risk reduction for SRE (risk ratio: 0.64; 95 % CI, 0.484–0.845; $p = 0.002$). These results promoted the approval of zoledronic acid as the first osteoclast targeted bone therapy for men with bony metastatic CRPC.

Zolendronic Acid—Castration Sensitive Metastatic Prostate Cancer

The Cancer and Leukemia Group B (CALGB) 90202 (Alliance) trial was a phase III randomized control trial designed to evaluate the safety and efficacy of early administration of zoledronic acid in men with castration sensitive (ADT naive) prostate cancer with metastases to bone [97]. The study aimed to enroll 680 men who had initiated ADT within six months of study entry to zoledronic acid (4 mg every 4 weeks) or placebo. The primary endpoint was time to first SRE defined by radiation to bone, pathologic fracture, spinal cord compression, surgery for bone or death due to prostate cancer. The study was terminated prematurely with 645 patients due to withdraw of sponsor support. The study analysis was planned at 470 SREs, but only 299 were recorded. Median time to first SRE was 31.9 month in the treatment group and 29.8 months with placebo (HR: 0.97; 95 % CI 0–1.17; $p = 0.39$). Overall survival was similar between groups (HR: 0.88; 95 % CI 0.7–1.12; $p = 0.29$) and rates of adverse events were no different as well. The trial conclusion did not support the use of early zoledronic acid prior to castration resistance. However, subgroup analysis of 82 men with previous SRE indicated significant delay in second SRE with zoledronic acid (31.9 vs. 17.6 months; HR: 0.56, 95 % CI 0.31–1.02; $p = 0.054$).

Pamidronate—Metastatic CRPC

In a combined analysis of two multicenter randomized, placebo-controlled trials (CGP 032 and INT 05) 350 men with CRPC and symptomatic bone metastases were given 90 mg pamidronate or placebo every 3 weeks (total 27 weeks) [98]. Endpoints included pain, analgesic use and SRE. Pool analysis revealed no difference in pain scores, analgesic use, SRE or survival. Biological plausibility for the results of this study is likely related to the decreased potency of pamidronate and advanced (symptomatic) stage of these patients.

Clodronate—Metastatic CRPC

The National Cancer Institute of Canada Clinical Trials Group (NCIC CTG) PR.6 study assessed the benefit of intravenous clodronate in patients with symptomatic, metastatic CRPC actively receiving chemotherapy. This placebo-controlled trial randomized 209 men being treated with mitoxantrone and prednisone to received

IV clodronate (1500 mg every 3 weeks) or placebo [99]. The primary endpoint was palliative response based on 50 % improvement in pain index scores, analgesic use and quality of life questionnaires. Palliative response was reported in 46 % of patients receiving clodronate and 39 % of patients receiving placebo ($p = 0.54$). The results suggest that the addition of clodronate to mitoxantrone plus prednisone does not improve palliation or quality of life.

Clodronate—Castration Sensitive Metastatic Prostate Cancer

The Medical Research Council (MRC) PR05 study randomized 311 men with castration sensitive prostate cancer with bony metastases to oral clodronate (2080 mg daily) or placebo [100]. All men were either being initiated or responding to primary ADT when enrolled and continued ADT through the trial. The primary endpoint was symptomatic progression of metastatic disease or disease specific death. A median follow-up of 59 months was reported in the clodronate group who had a non-significant trend in prolonged bone progression-free survival (HR: 0.79, 95 % CI 0.61–1.02, $p = 0.066$) and overall survival (HR: 0.80, 95 % CI 0.62–1.03, $p = 0.082$) [100]. However, long-term analyses of overall survival confirmed a significant benefit in the clodronate group compared to placebo at median follow up of 11 years (HR: 0.77, 95 % CI 0.60–0.98, $p = 0.032$) [101].

Denosumab

The Amgen Inc. sponsored denosumab protocol 20050103 (NCT 00321620) was a multinational, multicentered, randomized, double-blinded, controlled trial evaluating the efficacy of denosumab versus zoledronic acid in men with CRPC and bone metastases. The study randomized 1901 men with CRPC to denosumab (120 mg subcutaneously every 4 weeks) or zoledronic acid (4 mg intravenously every 4 weeks) [102]. The primary endpoint was time to first SRE (pathological fracture, radiation or surgery to bone or spinal cord compression) while on study. The primary objective was assessing for noninferiority of denosumab to zoledronic acid. The secondary objective was demonstrating superiority of denosumab along with comparative tolerability and safety.

Important exclusion criteria included creatinine clearance of less than 0.5 mL/second, current or previous bisphosphate therapy, planned radiation or surgery to bone, significant hypo- or hypercalcemia and life expectancy less than 6 months. It was recommended that all patients receive daily dose of calcium and vitamin D.

Preliminary study reports revealed that denosumab was superior to zoledronic acid in delaying time to SRE. Median on-study duration was 12.2 months in denosumab arm and 11.2 in the zoledronic acid arm. Median time to SRE on denosumab was 20.7 months versus 17.1 months on zoledronic acid (HR: 0.82, 95 % CI 0.71–0.95; $p = 0.0002$) non-inferiority ($p = 0.008$ for superiority). Overall survival and time to disease progression were similar between the two groups. For adverse events, more hypocalcemia was noted in the denosumab group (13 % vs.

6 %, p = 0.001), and osteonecrosis of the jaw was indifferent and infrequent between groups.

Radium 223

In 2012 results from the Alpharadin in Symptomatic Prostate Cancer trial (ALSYMPCA) phase-III efficacy study were revealed [58, 59]. Ra-223 became the first radiopharmaceutical to demonstrated overall survival benefit for metastatic prostate cancer. The study randomized 922 CRPC patients with ≥2 symptomatic bone metastases and no visceral metastases to receive 6 treatments every 4 weeks of Ra-223 or placebo. The primary endpoint was survival at 3 years. Secondary endpoints included time to first SRE, time to alkaline-phosphatase progression and response, time to PSA progression, safety and quality of life. Median overall survival in the Ra-223 group was superior to the placebo group by 2.8 months (14 vs. 11.2 months respectively, HR 0.659, 95 % CI: 0.552–0.875, p = 0.0019). The study was terminated early in recognition of the significant treatment benefit. Additionally, there were fewer SREs and time to first SRE was delayed in the treatment arm compared to placebo (13.8 vs. 8.4 months respectively, HR 0.610, 95 % CI 0.461–0.807, p = 0.0005).

Bone Targeted Therapies for Prevention of SRE or Metastasis—Table 14.2

Denosumab

Denosumab Versus Placebo for Prevention of Fracture in Non-metastatic CRPC

The Hormone Ablation Bone Loss Trial (HALT) 138 study enrolled 1468 men with non-metastatic prostate cancer receiving ADT [103]. All men were considered high risk for fracture based on age ≥70, BMD T-score <−1.0 or history of osteoporotic fracture. The trial was a double-blinded, multicentered and randomized to denosumab 60 mg subcutaneously every 6 months for 24 months versus placebo. Primary endpoints included BMD change in lumbar spine at 24 months. Secondary endpoints were new vertebral fractures or change in BMD at other joints.

Compared to placebo, BMD in the denosumab group significantly increased in the lumbar spine (5.6 %), total hip (4.8 %, femoral neck (3.9 %), and distal third of the radius (5.5 %). Denosumab was also associated with a decreased incidence of new vertebral fracture at 36 months (1.5 % vs. 3.9 %; RR 0.38, 95 % CI 0.19–0.78, p = 0.006). Rates of adverse events were similar between groups.

Denosumab and Bone Metastatic Free Survival in Non-metastatic CRPC

The strong clinical evidence for denosumab delay in SRE for metastatic CRPC patients and biologic plausibility of denosumab impact on the bone

Table 14.2 Bone targeted preventative therapies for prostate cancer (metastatic and non-metastatic)

	Trial	*n*	Study population	Arms	Primary endpoint	Results	Comments
Denosumab	HALT [103]	1486	Non-metastatic PC receiving ADT	60 mg denosumab every 6 months versus placebo for 3 years	BMD changes in lumbar spine at 24 months	Increased BMD by 5.6 %	Decreased cumulative incidence of vertebral fractures by 62 % (RR: 0.38, $p = 0.0006$)
	Denosumab 147 [104]	716	Non-metastatic PC with rising PSA	120 mg denosumab every 4 weeks versus placebo	Bone metastases-free survival	29.5 versus 25.2 months; (HR 0.85, $p = 0.028$)	No differences on OS, no effect on quality of life or pain
Bisphosphonates	MRC PR04 [107]	508	Non-metastatic PC within 3 years of local therapy ± ADT	2080 mg clodronate daily	Time to symptomatic bone metastases or death	No improvement in bone-metastases free survival (HR: 1.22, $p = 0.23$)	Median survival 9.5 years, no difference in OS survival
	Zometa 704 [106]	389	Non-metastatic CRPC	4 mg IV zoledronic acid versus placebo every 4 weeks	Time to first metastasis	Failed to meet accrual due to low event rate	Median time of bone metastasis-free survival was 30 months
	ZEUS [105]	1433	High-risk localized PC	4 mg IV zoledronic acid every 3 months versus observation	On-study development of metastasis	17 % versus 17.1 % at 4 ± 0.5 years follow up ($p = 0.95$)	No support for use of zoledronic acid for prevention of metastases in high-risk PC

ADT Androgen depravation therapy, *CRPC* castration resistant prostate cancer, *HR* hazard ratio, *mCRCP* metastatic castration-resistant prostate cancer, *OS* overall survival, *PC* prostate cancer, *RR* relative risk, *SRE* skeletal related event

microenvironment encouraged investigation of RANKL inhibition for prevention of prostate cancer metastasis. The Amgen Inc. 147 trial was a phase III, multicenter, double-blind, randomized, placebo controlled study of non-metastatic prostate cancer patients with rising PSA (≥8 mg/mL and/or doubling time ≤10 months) [104]. The study randomized 716 patients to received denosumab 120 mg subcutaneous every 4 weeks and 716 patients to placebo. The primary endpoint was bone-metastasis-free survival (symptomatic or asymptomatic). Patients received standard therapy at the choice of the treating provider.

The final analysis demonstrated a median increase by 4.2 months for bone-metastasis-free survival vs. placebo (29.5 vs. 25.2 months; HR 0.85, 95 % CI 0.73–0.98, $p = 0.028$). Denosumab significantly delayed the time to first bone metastases (32.2 vs. 29.2 months; $p = 0.032$). However, no difference was noted in overall survival (43.9 vs. 44.8; $p = 0.91$). There was no effect on quality of life or pain. Adverse event rates were similar between groups, but patients on denosumab were more likely to experience osteonecrosis of the jaw and hypocalcaemia.

This was the first study to demonstrated that changing the bone microenvironment with bone preservation can delay metastatic disease. However, the extent of clinical benefit is questionable. The marginal benefit of only 4.2 months to delay metastasis without impact on survival was not sufficient to gain approval of an FDA indication for delaying metastatic disease. The most valuable use of denosumab in current treatment paradigms for non-metastatic disease will need further investigation.

Bisphosphonates

Zoledronic Acid Versus Standard Therapy—Prevention of Bone Metastasis

The Zometa European Study (ZEUS) assessed the utility of zoledronic acid in preventing bone metastases in high-risk, non-metastatic localized prostate cancer [105]. The study randomized 1433 patients to standard therapy with or without zoledronic acid 4 mg IV every 3 months for 48 months. Over one-third of patients were not on ADT. Eligible patients were required to have at least one high-risk feature including PSA ≥20 mg/mL, node positive disease or Gleason 8–10 disease. On-study development of any bone metastasis was the primary endpoint. A total of 1393 patients randomized were used for intention-to-treat analysis. At 4 ± 0.5 years 17.1 % of the zoledronic acid group and 17 % of the control group had diagnosed metastases (chi squared test, $p = 0.95$). At a median follow-up of 4.8 years the Kaplan-Meier estimates rate of metastasis for the whole group was 14.7 % for the zoledronic acid group and 13.2 % in the control group. This was not significantly different (log-rank: $p = 0.65$). Additionally, there was no difference in overall survival ($p = 0.71$). In conclusion, zoledronic acid every 3 months was ineffective for the prevention of bone metastases in high-risk, non-metastatic prostate cancer.

Zoledronic Acid Versus Placebo—Prevention of Bone Metastasis

The Zometa 704 trial was a randomized, double-blind, placebo-controlled study conducted to assess the effects of zoledronic acid on the time to first metastasis in men with non-metastatic CRPC. Patients were required to have PSA progression despite ADT. Patients were randomized to zoledronic acid (4 mg IV every 4 weeks) or placebo. Target accrual was 991 patients. Three years after beginning the trial in September 2002, 389 patients had been enrolled and the study was placed on hold due to lower than expected event rate. This precluded evaluation for efficacy. Median time of bone-metastasis-free survival was 30 months. Baseline PSA and PSA velocity did independently predict decreased time to first bone metastasis, bone-metastasis-free survival and overall survival. Although this study did not provide evidence for the efficacy of zoledronic acid in this particular setting, the observations facilitated early identification of men at high risk for developing bone metastatic disease [106].

Clodronate Versus Placebo—Non-metastatic CRPC

The MRC PR04 study evaluated the utility of adjuvant clodronate bisphosphonate therapy to improve symptomatic bone-metastasis-free survival in patients with non-metastatic prostate cancer patient at high risk of developing metastatic disease [107]. The study included 508 men within three years of diagnosis and local therapy for prostate cancer with external beam therapy, external beam with ADT or primary ADT as standard treatment. The men were randomly assigned to oral clodronate (2080 mg daily) versus placebo for 5 years. The primary endpoint was time to symptomatic bone metastases or death due to prostate cancer. At a median follow up of 10 years there was no evidence of significant improvement in bone-metastases-free survival in the clodronate treatment group (HR = 1.22, 95 % CI 0.88–1.68, $p = 0.23$). There was no difference in overall survival (median survival 9.5 years) and there were more adverse events in the clodronate group (GI intolerance and increased LDH). Clodronate did not change the natural history of non-metastatic prostate cancer. These results are in contention with the use of clodronate in castration-sensitive metastatic disease described previously [101].

SERMs—Toremifene Versus Placebo for Men Receiving ADT for Prevention of Fractures

Known as toremifene protocol G300203, this study evaluated the effects of a selective estrogen receptor modulator on incidence of fractures in men receiving ADT within a 2-year period [78]. Study design was double-blind, placebo controlled phase 3 including 646 receiving ADT for prostate cancer randomized to toremifene 80 mg by moth daily vs. placebo. The study included men >50 years old receiving ADT for 6 months or more or intermittent ADT for 12 or more months prior to enrollment. Men were at any stage in treatment of prostate cancer. Primary endpoint was new vertebral fractures with secondary endpoint of fragility fracture, BMD loss and lipid changes.

Men receiving toremifene had significantly fewer vertebral fractures (2.5 % vs. 4.9, respectively) with relative risk reduction of 50 % (95 % CI 1.5–75.0, $p = 0.05$). Toremifene also significantly increased BMD at the lumbar spine, hip and femoral neck by about 2 % in all categories. Additionally, lipid changes in the treatment group were beneficial.

Non-bone Targeted Therapy

Novel Anti-androgens

A phase III clinical trial randomizing 1195 men previously treated with docetaxel to abiraterone + prednisone or placebo + prednisone. After a median follow-up of 12.8 months there was an overall survival benefit from abiraterone (14.8 vs. 10.9 months, respectively; HR 0.65, 95 % CI 0.54–0.77, $p < 0.001$) [108]. Other phase II study has reported statistically significant improvements in median time to opiate use and improved pain control in chemotherapy-naïve metastatic CRPC patients [109].

The phase III, double-blind AFFIRM trial randomized 1199 men with CRPC who had failed docetaxel therapy to enzalutamide or placebo at a ratio of 2:1. The trial was stopped after a planned interim analysis demonstrated an overall survival advantage for the enzalutamide group (18.4 vs. 13.6 months respectively; $p < 0.0001$). Furthermore the superiority of the enzalutamide group was confirmed for all secondary end points (reduction in PSA, soft tissue response rate, time to PSA progression, radiographic progression free survival and time to first skeletal related event) [84]. Furthermore, evidence of significantly delayed time to pain progression and overall improvement in health-related quality of life assessments were reported in the enzalutamide group [84].

Conclusions

Bone health preservation is a critical element in comprehensive management of prostate cancer. Methods to reduce associated treatment-related and/or disease related bone weakness range from simple lifestyle modifications to complex osteoclast-targeted agents. A growing pool of evidence suggests that pharmacologic bone-targeted therapies prevent many of the morbidities associated with bone metastases and may enhance overall survival in some situations. The RANKL signaling pathway has proven to be an excellent therapy target and inhibition has demonstrated superior clinical results in men on ADT with mCRPC, delaying time to skeletal related events by over 3 months in comparison to zoledronic acid. Furthermore, RANKL inhibition has also demonstrated a preventative role in preserving bone mineral density and improving metastasis-free survival. As the treatment landscape continues to change, additional investigation is required to better characterize the optimal dosing, duration and identify those who will benefit

most from preventative treatment. Lastly, this book chapter does not address the cost of such medications. Cost considerations should incorporated into future research to best identify medications that provide the have highest clinical value.

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Novel Therapies in Castration-Resistant Prostate Cancer

15

Tyler Lash and Rhonda L. Bitting

Introduction

Over the past several years, there has been significant progress in treatments and outcomes in castration-resistant prostate cancer (CRPC), with multiple new drugs with varying mechanisms gaining U.S. FDA approval. Despite these improvements, treatment resistance emerges within 1–2 years and CRPC remains incurable. Novel approaches are needed. With the clinical use of more potent androgen pathway inhibitors, the emergence of androgen-independent phenotypes is predicted to rise. To address this, agents targeting stemness, cellular differentiation, and invasion will be needed, likely in combination with current therapies that target the more differentiated and androgen-driven bulk of disease. In preclinical models, combination treatment approaches have resulted in durable remissions [1]. Therefore, rational combination therapies, based on the knowledge of resistance pathways and of immunologic escape, will likely be the most effective way to eradicate CRPC.

Here we discuss a selection of promising therapies in CRPC, which utilize a variety of mechanisms to combat the disease. First, we describe the role of immunotherapy, specifically with immune checkpoint blockade. We then discuss androgen-directed therapies, with a focus on those therapies likely to be effective

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despite androgen receptor variants. Finally, we describe therapies that are predicted to be effective for more de-differentiated disease such as chemotherapy and treatments targeting stemness pathways.

Immunotherapy

Immunotherapy is an attractive approach for refractory cancers, including prostate cancer, as exemplified by the approval of sipuleucel-T and anticipated approval of a poxvirus-based PSA targeted vaccine, PSA-TRICOM, for metastatic CRPC [2, 3]. The recent introduction of immune checkpoint blockade with antibodies to cytotoxic T lymphocyte-associated antigen 4 (CTLA-4) and to the programmed cell death protein 1 (PD-1) pathway, which includes the PD-1 receptor and its ligands, PD-L1 and PD-L2, has been an important advance in the treatment of advanced cancer. Both the CTLA-4 and PD-1 receptors are normally expressed on activated T-cells, and when the receptor encounters its ligand, the T-cell is inactivated. Tumor cells and tumor-associated lymphocytes may express these ligands and therefore evade host immune surveillance. Disruption of these immune checkpoints maintains and enhances effector T-cell responses, thereby leading to tumor regression [4, 5]. Immune checkpoint inhibition with the anti-CTLA-4 antibody, ipilimumab, and the anti-PD-1 antibodies, pembrolizumab and nivolumab, have revolutionized the treatment of melanoma and non-small-cell lung cancer. The combination of ipilimumab and nivolumab had a 61 % response rate in metastatic melanoma [6]. Nivolumab showed an improvement in OS of 3.2 months versus docetaxel in metastatic squamous NSCLC with progression on platinum based therapy [7]. There have also been promising results in kidney, bladder, head and neck cancers, and Hodgkin lymphoma. Limited activity has been observed in CRPC.

In phase I/II trials in CRPC, ipilimumab led to PSA decline >50 % in ≈15 % of cases [8, 9]. A phase III randomized study comparing ipilimumab to placebo after radiotherapy in metastatic CRPC was negative for the primary endpoint of OS, although subgroup analyses suggest that ipilimumab may provide an OS benefit for patients with favorable prognostic features and limited metastatic burden [10]. CA184-095 (NCT01057810) is a phase III trial assessing ipilimumab versus placebo in minimally symptomatic CRPC patients with favorable prognostic features, and this study will prospectively address the question as to whether there is a group of CRPC patients who will benefit from ipilimumab.

Recent findings have shown that patients progressing on enzalutamide have significantly more PD-L1/2^{+} dendritic cells and PD–1^{+} T-cells in their blood compared with those naïve or responding to treatment [11]. This suggests that PD-1 pathway inhibitors may have a role in treating CRPC. However, among 296 patients with advanced cancer in a phase I trial of nivolumab, there were 17 CRPC patients and no objective responses were reported [12]. Clinical trials of anti-PD-1 and anti-PD-L1 antibodies, combinations of immune checkpoint inhibitors, and combinations with other immunologically active agents, are in progress as shown in Table 15.1.

Table 15.1 Selected clinical trials of novel agents for men with CRPC

Agent	Description	Clinical trial
Immune checkpoint inhibitors		
Ipilimumab	Phase 1, 2 trial with abiraterone and prednisone in chemotherapy and immunotherapy-naïve patients with CRPC	NCT01688492
Ipilimumab	Phase 2 trial with ipilimumab with androgen suppression therapy in CRPC	NCT01498978
Ipilimumab	Phase 3 with ipilimumab versus placebo in chemo-naïve patients with CRPC	NCT01057810
Pembrolizumab	Phase 2 with pembrolizumab and enzalutamide in patients with CRPC who have progressed on enzalutamide	NCT02312557
Avelumab	Phase 1 anti PD-L1	NCT01772004
CT-011	Phase 2, anti PD-1 in combination with sipuleucel-T and cyclophosphamide	NCT01420965
AR-directed therapies		
Galeterone	Phase 3 with dual androgen synthesis and AR inhibitor galeterone versus enzalutamide in patients with AR-V7	NCT02438007
ODM-201	Phase 3, placebo-controlled in nonmetastatic CRPC	NCT02200614
VT-464	Phase 1,2 with dual androgen synthesis and AR inhibitor VT-464	NCT02012920
Stemness pathway inhibitors		
Vismodegib	Pharmacodynamic study of vismodegib in CRPC patients with metastatic lesions assessable for biopsy	NCT02115828
Sonidegib	Phase 1B in combination with docetaxel in CRPC	NCT02182622
Antibody-drug conjugates		
DSTP3086S	Phase 1, monoclonal antibody targeting STEAP-1 conjugated to monomethyl auristatin E in CRPC	NCT01283373
EC1169	Phase 1, conjugated monoclonal antibody targeting PSMA in patients with CRPC	NCT02202447
BIND-014	Phase 2, monoclonal antibody targeting PSMA and conjugated to docetaxel in CRPC	NCT01812746
Other		
Custirsen	Phase 3 cabazitaxel with or without custirsen in CRPC	NCT01578655

Androgen-Directed Therapy

Most CRPC is an androgen-driven process, as evidenced by the survival benefit shown with the androgen receptor (AR) antagonist enzalutamide and androgen synthesis inhibitor abiraterone. However, despite the success of these drugs, some patients will have no response and all patients will eventually progress despite these therapies. A phase 3 trial reported in 2012 showed that 21 % of patients had no decline in PSA with enzalutamide, suggesting primary resistance [13]. Possible mechanisms of resistance include AR signaling through mutations or splice variants, AR mutations

that interfere with enzalutamide binding, and AR-independent growth. Understanding the mechanisms of resistance will help to guide therapy and provide mechanisms for newer drugs.

In recent years, much progress has been made in the understanding of androgen receptor splice variants. Splice variants occur when there is truncation of the AR gene, which is caused by a premature stop codon prior to the region coding for the ligand binding domain. The AR isoform encoded therefore lacks the ligand-binding domain and remains constitutively active [14, 15]. Without the ligand-binding domain, current hormonal therapies are ineffective. AR-V7 is of particular interest because it is the most common splice variant, and its expression is increased approximately 20 fold in the castration-resistant, as opposed to the castration-sensitive, setting [16].

AR-V7 holds promise as the first predictive biomarker for CRPC. Circulating tumor cells were evaluated for the presence of AR-V7 via quantitative reverse-transcription PCR, with the hypothesis that detection of this variant would be associated with primary resistance to enzalutamide and abiraterone. 31 patients on enzalutamide and 31 on abiraterone were studied. Patients with detectable AR-V7 had inferior PSA response rates if receiving either enzalutamide (0 % vs. 52.6 %; P = 0.004) or abiraterone (0 % vs. 68 %; P = 0.004). Patients with detectable variant also had inferior progression-free survival [17]. This suggests that presence of the AR-V7 is associated with resistance to enzalutamide and abiraterone, potentially providing a biomarker for treatment selection. These findings have also encouraged the development of AR antagonists that inhibit at the N-terminal domain and would therefore not be affected by truncation and loss of the ligand-binding domain.

EPI-001 is a small molecule that targets the N-terminal domain of the AR with high specificity and blocks transcriptional activity of both full-length and variant AR. In preclinical models, EPI-001 inhibits androgen-dependent cell proliferation and tumor growth [18]. EPI-001 also reduces tumor growth in CRPC xenografts [19]. In both cell culture and mouse models, the combination of EPI-001 with docetaxel enhances the efficacy of chemotherapy [20]. A phase 1 study with EPI-001 has not yet been initiated, but the preclinical studies have laid the groundwork for testing both as a single-agent and in combination. Ideally, this drug will be further developed in parallel with a biomarker for AR-V7 detection, in order to select a patient group most likely to benefit.

There are several other promising AR pathway inhibitors currently under investigation. One such agent, galeterone, is a dual androgen synthesis and AR inhibitor and has shown safety and preliminary efficacy in early-phase clinical trials in CRPC [21]. There is suggestion that galeterone will have activity even in men with variant AR, therefore galeterone is being evaluated in a phase III study versus enzalutamide, specifically in patients with AR-V7 (NCT02438007). ODM-201 is another AR antagonist with promising safety and efficacy in CRPC [22]. Although it is very similar in mechanism to enzalutamide, in the preclinical setting ODM-201 has activity against both full-length and variant AR [23], and a phase III study is

underway in CRPC (NCT02200614). Other agents in development are similar in action to enzalutamide (ARN-509) or abiraterone (VT-464). While these agents may show sufficient activity to gain FDA-approval in CRPC, they will likely have similar patterns of resistance to those of enzalutamide or abiraterone, unless given in combination or with biomarker guidance.

Non-AR Targeted Therapies

Stemness Pathway Inhibitors

The Notch pathway is highly conserved and is required for the development of most tissues and organs. Abnormalities in the Notch signaling pathway have been implicated in prostate cancer progression. NOTCH-1, which is associated with a stem cell phenotype [24], is significantly upregulated in bone metastasis compared with the primary prostate tumor, suggesting that NOTCH-1 may be important for PC progression [25]. Further, prostate cancer cells that survive docetaxel exposure have activated Notch and Hedgehog signaling, suggesting that this pathway is activated as mechanism of treatment resistance [1]. Activation of Hedgehog or Notch signaling in CRPC patients suggests that treatment with agents that block these stemness pathways may be effective and is an active area of investigation.

PF-03084014 is a gamma-secretase inhibitor that inhibits Notch pathway signaling. This agent has been shown to have anti-tumor activity in prostate cancer murine models both alone and in combination with docetaxel [26]. Clinical studies have not yet started. With this and other similar agents, there is potential for significant stem-cell toxicity, therefore it is imperative that the agents be selective for tumor cells rather than normal hematopoietic cells.

The antifungal agent itraconazole is thought to block Hedgehog pathway signaling and has shown modest activity in CRPC patients [27]. Vismodegib is a Smoothened inhibitor that blocks signaling through the Hedgehog pathway and is FDA-approved for use in basal cell carcinoma, where mutations have rendered the pathway constitutively active. Aberrant Hedgehog signaling has been noted in prostate cancer and clinical data is emerging in CRPC. In prostate cancer cell line and murine studies, androgen deprivation leads to increased Hedgehog pathway signaling, and combination therapy with an AR antagonist plus a Hedgehog pathway inhibitor suppressed tumor growth far beyond the effects of either agent alone [28]. Although a prostate cancer stem cell has not been clearly identified and may or may not utilize the AR [29], strategies to target stemness pathways may be as important as AR targeting. However, given the central role of AR in prostate cancer, targeting AR in the context of additional therapies is likely essential. Itraconazole, vismodegib, and another Smoothened inhibitor sonidegib are under further investigation both as single agents and in combination for CRPC [30].

Antibody-Drug Conjugates

Over the past couple of decades, the FDA has approved more than a dozen monoclonal antibodies for cancer treatment. Some are "naked," such as alemtuzumab, trastuzumab, and ipilimumab. They exert their effects by attaching to antigens and blocking their ability to elicit signaling or mark them for destruction. Others are linked to cytotoxic substances such as chemotherapy or radiation and are referred to as conjugated monoclonal antibodies. These have been met with much success in solid tumors as well as hematologic malignancies. Trastuzumab emtansine, also known as T-DM1, is a HER2-targeted antibody, trastuzumab, conjugated to the microtubule-inhibiting agent DM1. T-DM1 showed an improvement in OS of approximately 6 months when compared to lapatinib and capecitabine as second-line therapy for advanced HER2 positive breast cancer [31]. Brentuximab vedotin, a CD-30 targeting antibody linked to the antitubulin agent monomethyl auristatin E, was granted accelerated approval after a phase II trial showed an objective response rate of 73 % and median duration of 6.7 months for relapsed Hodgkin disease [32].

Investigators are also hoping for success with conjugated monoclonal antibodies in prostate cancer. Currently the most commonly targeted antigen is prostate specific membrane antigen (PSMA), which is highly expressed in prostate epithelial cells and upregulated in prostate cancer. It can also be found in the neovasculature of other solid tumors but is minimally expressed in most normal tissue [33]. First generation drugs utilizing PSMA for selective delivery are currently undergoing clinical trials (see Table 15.1).

BIND-014 is a PSMA-targeting antibody attached to docetaxel and was shown to have activity in 9 of 28 heavily pretreated patients with various tumor types (including a partial response in 1 with prostate cancer) in a phase 1 trial [34]. There is an ongoing phase 2 trial evaluating its efficacy in patients with metastatic CRPC (NCT01812746). PSMA ADC is another PSMA-targeting antibody, attached to the microtubule disrupting agent monomethyl auristatin E, and was shown to have activity in approximately 50 % of patients in a phase 1 trial of taxane-refractory CRPC [35]. A phase 2 study of PSMA ADC also showed disease response, as evidenced by declines in circulating tumor cells, declines in PSA, and radiologic response (79 % with stable disease) [36]. MLN2704 also targets PSMA and is conjugated to the antimicrotubule drug maytansinoid-1. It was shown to have activity in a phase 1 trial of patients with CRPC, including 2 patients having >50 % reduction in PSA [37].

Six transmembrane epithelial antigen of the prostate (STEAP-1) is predominantly expressed in the prostate tissue and provides another potential target. DSTP3086S is an antibody directed at STEAP-1 and conjugated to monomethyl auristatin E. A phase 1 trial showed a tolerable safety profile with anti-tumor activity as evidenced by reductions in circulating tumor cells or PSA [38]. Enrollment in the expansion cohort is ongoing.

Antibody-drug conjugates are also under consideration as part of combination therapy. Androgen suppression increases expression of PSMA, which provides a mechanism by which conjugated monoclonal antibodies could be synergistic with anti-androgen therapy [39]. A recent trial evaluating PSMA ADC with enzalutamide and abiraterone showed synergy between these agents. In this study, antiproliferative activity and PSMA expression were evaluated in two prostate cancer cell lines (LNCaP and C4-2) exposed to antiandrogens alone or in combination with PSMA ADC. In androgen-dependent LNCaP cells, the antiandrogens were shown to inhibit proliferation, upregulate PSMA, and synergize with PSMA ADC. In the androgen-independent C4-2 cells the antiandrogens did not inhibit proliferation but did upregulate PSMA expression and synergized with PSMA ADC. PSMA ADC was also shown to synergize with the PI3K/mTOR inhibitor rapamycin [40].

Antibody-drug conjugates are appealing in that they can provide more accurate, effective delivery of drug while providing less systemic toxicity than more traditional therapies. This mechanism becomes even more interesting when considering that the addition of ADT could enhance the delivery of drug by upregulation of its target antigen. Further trials are needed to confirm this mechanism as well as identify their appropriate place in the algorithm of treatment.

Antisense Oligonucleotides

Antisense therapy is aimed at a particular gene known to cause pathology. A sequence of nucleic acid (antisense sequence) is synthesized which is complementary to the mRNA (sense sequence) of the gene of interest. By binding the mRNA, it turns off the gene and prevents translation. Although few drugs in this category have been FDA-approved, the mechanism is intriguing and there are many drugs utilizing this mechanism under development. At the time of this writing, there are 71 studies listed on clinicaltrials.gov for antisense oligonucleotides.

Clusterin is a cytoprotective chaperone protein that can be activated by stress and protect cells from apoptosis [41]. OGX-011, now called custirsen, is an antisense oligonucleotide that is complementary to the clusterin mRNA translation initiation site and inhibits its translation [42]. A phase I study showed the drug could safely be administered with docetaxel [43]. A phase II study then showed a trend towards improved outcomes with the addition of OGX-011 to docetaxel, with a progression-free survival of 23.8 versus 16.9 months with docetaxel monotherapy [44]. The phase III SYNERGY trial was performed to evaluate if OGX-011 improved survival when added to first-line standard therapy docetaxel and prednisone. Unfortunately there was no statistical difference in median overall survival (OS). However, a post hoc analysis of the SYNERGY trial presented at the 2015 ASCO conference showed a significant survival benefit in patients with poor prognosis, with a median OS of 17 months versus 14 months [45]. OGX-011 is also being evaluated in CRPC in a phase III trial in combination with cabazitaxel that has completed accrual (NCT01578655).

Conclusions

Advances in the understanding of the biology and pathophysiology of prostate cancer have led to exciting, rationally designed drugs and treatment approaches. By applying lessons learned from other tumor types, CRPC patients will hopefully continue to benefit from promising and novel therapies. For example, further understanding of the role of the immune system in cancer progression has brought immunotherapies such as immune checkpoint inhibitors into the forefront for many advanced cancers, including prostate. Likewise, antibody-drug conjugates, which can potentially improve the delivery of drug to target cells as well as decrease systemic toxicity, may have a place in the prostate cancer treatment landscape. Additional understanding of androgen receptor biology, such as the emergence AR variants that lack the ligand-binding domain, has allowed for development of new drugs directed towards old targets, while simultaneously, numerous therapies with novel mechanisms are also being developed.

Although progress is being made, CRPC lags behind other advanced malignancies in terms of biomarker development. Biomarkers currently used clinically in CRPC, such as PSA, are prognostic but do not guide treatment choices. Circulating tumor cells are also prognostic in CRPC [46], and there is hope that CTCs can eventually be used as predictive biomarkers as well, as described above with the detection of AR variants. Advanced sequencing techniques, both on tumor tissue and circulating cells or DNA, will continue to offer opportunities for biomarker discovery and development. Ongoing trials of active systemic therapies with prospectively embedded biomarker studies will be essential before these can be used for definitive clinical decision-making. Moving forward, biomarkers must be studied rigorously in parallel with drug development.

Only a select number of novel therapies for CRPC are described above. As CRPC is parsed into genetically and phenotypically distinct subtypes [47], treatments will be further customized. For example, 40 % of CRPC with neuroendocrine features has overexpression of aurora kinase A, compared to only 5 % of classic CPRC, suggesting a role for aurora kinase inhibition in neuroendocrine disease [48]. PARP inhibitors will likely be effective in BRCA1/2 mutant prostate cancers [49]. Due to the prevalence of PI3K pathway abnormalities in CRPC, there are multiple PI3K/Akt/mTOR pathway inhibitors currently in clinical trials. Early data suggests that these agents will be most effective when given in combination and that biomarkers are needed to select appropriate patients for treatment [50]. Aurora kinase inhibition, PARP inhibition, and PI3K pathway inhibition are only a few examples of treatments that may be considered for specific subgroups of CRPC patients, if these subgroups can be consistently identified.

There is also more to learn about the drugs already FDA-approved for CRPC. Currently there is no standard for the sequencing of therapy, and abiraterone, enzalutamide, sipuleucel-T, alpharadin, and docetaxel are all category 1 recommendations for first-line treatment of metastatic CRPC. As novel therapies are

incorporated into the CRPC landscape, it will be crucial to consider the context and to develop biomarkers of response or resistance to guide treatment decision-making.

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