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Management of Castration Resistant Prostate Cancer

Humana Press

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ISSN 2197-7194 ISSN 2197-7208 (electronic)
ISBN 978-1-4939-1175-2 ISBN 978-1-4939-1176-9 (eBook)
DOI 10.1007/978-1-4939-1176-9
Springer New York Heidelberg Dordrecht London

Library of Congress Control Number: 2014945340

Printed on acid-free paper

Humana Press is a brand of Springer
Springer is part of Springer Science+Business Media (www.springer.com)

The opportunity to serve as co-editor of this book is the fruit of over 25 years of working in the field of prostate cancer. I would like to thank the patients who have taught me how complex this disease really is. As a clinical researcher I am especially grateful to those patients who accepted to be included in clinical trials. They have allowed me to witness some of the most extraordinary results I have seen in my career. These patients helped discover new treatment options that improve and prolong the lives of others. I am also extremely grateful to my clinical colleagues and co-researchers who have contributed to improving our understanding and management of prostate cancer. They have truly helped patients living with prostate cancer but we still have much to do.

Finally, I thank and dedicate this book to my amazing wife Rachel and the four beautiful children she has given me: Genevieve, Julien, Veronique and Simon. Their love has made my career and life complete.

Fred Saad

Over the past several years I had the privilege to conduct multiple clinical trials in patients with prostate cancer, some established new standards of care. This work is dedicated to all patients (and their families) who entrusted their lives to me and for those who so courageously agreed to participate in clinical trials. Without them we would never be where we are now. This book is also dedicated to my colleagues, clinical and laboratory scientists, who have devoted their lives to improve the outcomes of cancer patients.

To my wife Jannie (my soul mate), thank you for your support, patience, and understanding during all these years. I am grateful to the Universe for leading me through my current path of life and for the precious gifts that I have been granted and tried to cherish all these years. I pray and hope that one day, my patients and I together, can witness the cure of this devastating disease. To all I transmit what gives me strength and hope in my daily life: "Never despair! Never! It is forbidden to give up hope" (Rebbe Nachman of Breslov 1772–1810).[1]

Mario A. Eisenberger

[1] The Empty Chair; Finding Hope and Joy. Timeless Wisdom from a Hasidic Master, Rebbe Nachman of Breslov (adapted by Mykoff M and the Breslov Research Institute). Jewish Lights Publishing, Woodstock, Vermont, 2005, p. 110.

Preface

Prostate cancer is one of the most fascinating challenges we face in oncology. Controversy about screening and over treatment has become the focus of attention in almost every developed country in the western world. There has been an explosion of new cases and an abundance of what many consider to be "insignificant cancers" diagnosed. This has led large segments of the lay population, as well as in the medical profession, to forget that prostate cancer is still one of the top three causes of cancer-related death in the western world. For almost all men who die of prostate cancer, castration-resistant disease will be the cause of death. Until recently new therapeutic options that make a difference were so limited that most men did not receive any treatment beyond androgen deprivation therapy and supportive care. The last few years have seen the field go from rags to riches in terms of effective options and research in the biology of CRPC is leading to exciting new therapeutic targets. Given all the new data that is critical in understanding how we got here and where the field is going it was time to gather some of the most important players in the field and put it all together in an up-to-date book dedicated to castration-resistant prostate cancer. The book reviews new data about the molecular biology of CRPC as well as the staging procedures and prognostic factors that define CRPC. An in-depth review of proven therapeutic options is provided that includes hormonal-based therapies, bone targeted therapies, immunotherapy, and chemotherapy. Combination therapy and novel targeted approaches presently under investigation are also reviewed. Finally the book concludes with an evidence-based management strategy based on present-day knowledge and international guidelines.

It was an honor to have had the opportunity to serve as the editors of this book. With the incredible contributions by our colleagues who accepted to write their respective chapters we certain that this book will serve as a useful resource for physicians and researchers dealing with, and interested in, this challenging state of prostate cancer. We also would like to dedicate this book to all patients with prostate cancer and hope that this will contribute to further improve their chances of survival and enhance their quality of life.

We would like to thank to the team at Springer for realizing the importance of the subject matter and a special thanks to Mr. Michael Griffin for his constant support in seeing this project become a reality.

Montreal, QC, Canada — Fred Saad, MD, FRCS
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Part I

Castrate-Resistant Prostate Cancer: Clinical and Biological Considerations

1 Introduction—Castration Resistant Prostate Cancer: A Rapidly Expanding Clinical State and a Model for New Therapeutic Opportunities

Mario A. Eisenberger and Fred Saad

Substantial progress has been made in the clinical aspects, biology and treatment of prostate cancer especially in patients with advanced disease who show evidence of disease progression following gonadal suppression. This book focuses on the latest developments in the biology, clinical and therapeutic aspects of the castration resistant state. This state is defined as patients who present with evidence of disease progression after initial gonadal suppression. In the past these patients were categorized as having hormone-refractory prostate cancer. During the past decade the field has witnessed an extraordinary change in much of the concepts and definitions about the biology of this state which have resulted in major improvements in outcomes. Recent approval of new treatments improved survival and the quality of life of patients with advanced prostate cancer. Evolving management patterns have undoubtedly impacted in the clinical landscape of the disease. Routine measurement of prostate-specific antigen (PSA) has profoundly affected virtually all clinical aspects of prostate cancer. A sharp increase in the incidence of age-adjusted prostate cancer and the proportion of patients with early stages of the disease at the time of diagnosis (stage migration) has coincided with the advent of widespread PSA testing [1, 2]. In a relatively short period of time (only two decades), there has been a categorical shift in the extent of disease at the time of the initial diagnosis of all stages of prostate cancer. The proportion of patients with clinical evidence of regional and distant metastasis at the time of initial diagnosis has decreased dramatically. Routine reliance on serum PSA testing in the treatment decision processes has also profoundly influenced the clinical landscape of the disease. It is generally felt that conventional staging grouping (TNM staging) does not adequately represent the clinical status of relapsed prostate cancer [3]. The outcome of advanced prostate patients has improved in all aspects compared to the past (pre PSA era) experience as a result of a lead time effect (stage migration) and survival/quality of life improvements with the addition of new effective treatments to our therapeutic armamentarium.

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The Dynamics and Natural History of Metastatic Prostate Cancer and Castration Resistant Disease

The chapter on evolution of clinical states and the castration resistant clinical paradigm (Chap. 2 by Zhang and Armstrong) articulates the dynamics

F. Saad and M.A. Eisenberger (eds.), *Management of Castration Resistant Prostate Cancer*, Current Clinical Urology, DOI 10.1007/978-1-4939-1176-9_1,

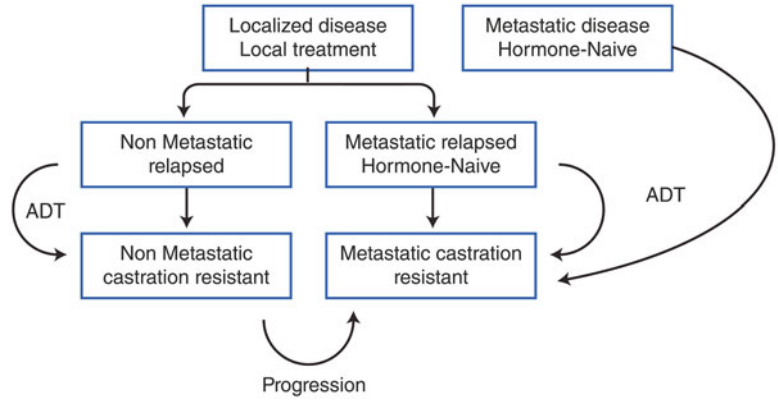

Fig. 1.1 Management-based dynamic progression of prostate cancer and the mCRPC state. *ADT* androgen deprivation treatment

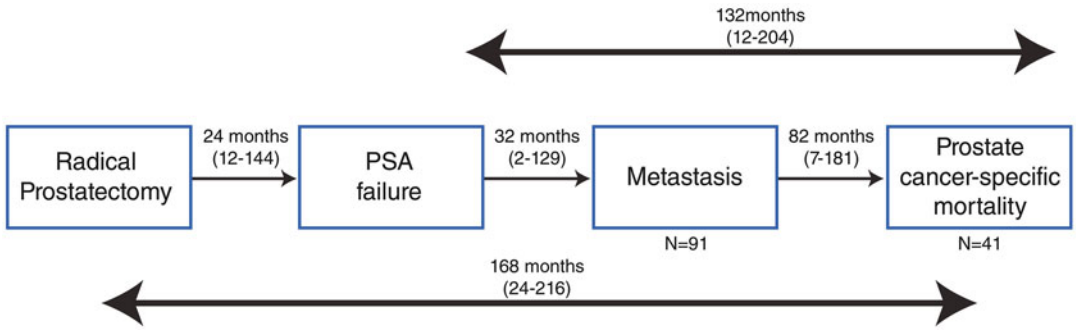

Fig. 1.2 Survival of metastatic prostate cancer patients diagnosed in the context of a close follow-up after biochemical relapse: The Johns Hopkins Experience (from Makarov et al. [4])

of all clinical models in prostate cancer with discussions around clinical features and biomarker data that may further define this heterogeneous group of patients and potentially bring some therapeutic sense into the era of individualized treatment strategies. Appropriate definition of the clinical course and characterization of clinical/laboratory landmarks (new markers, circulating tumor cells, new imaging techniques, etc.) is critical for moving the field forward towards a more logical approach for definition of outcomes and the selection/testing of promising therapeutic modalities for patients with CRPC.

The clinical spectrum of castration resistant prostate cancer has evolved in parallel with the emerging treatment patterns in clinical practice (Fig. 1.1). In hormone naïve, biochemically relapsed patients, routine serial PSA testing coupled with systematic clinical and radiological follow-up has resulted the diagnosis of metastasis and consequently with survival outcome figures that exceed by many folds the historical experience. This is well illustrated by the Johns Hopkins Hospital experience in patients who relapse biochemically after local treatment and are subsequently monitored with frequent serial PSA determinations and routine yearly bone scans. These patients received no systemic treatment until they developed evidence of metastatic disease. The prostate cancer specific survival figure in patients who developed M + disease in this setting was 82 months (Fig. 1.2). All patients in this series have limited metastatic disease and have other favorable prognostic features such as normal hemoglobin levels and no symptoms attributable to metastatic disease [4].

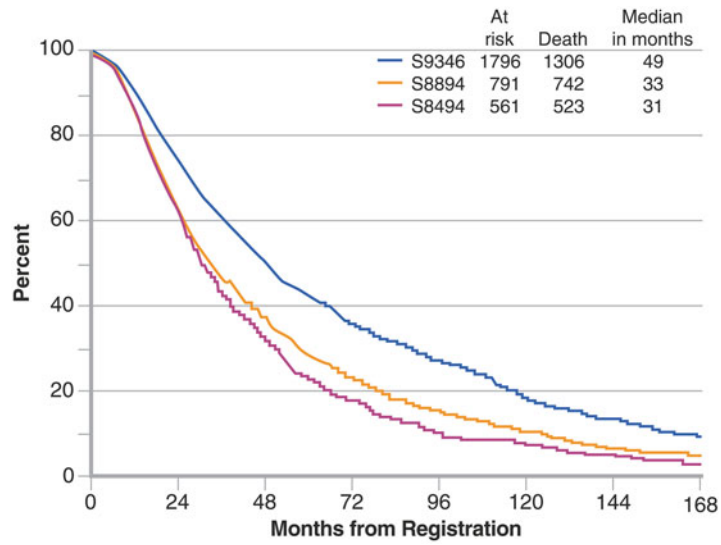

Fig. 1.3 Kaplan–Meier survival of newly diagnosed metastatic, hormone naïve, prostate cancer patients in three studies conducted by the Southwest Oncology Group (reprinted from Tangen et al. [6])

An increasing proportion of patients with relapsed prostate cancer receive androgen deprivation prior to the development of metastatic disease and eventually demonstrate evidence of advancing disease initially manifested by rises of serum PSA levels without any other clinical/radiological evidence of disease. This subset of patients is classified as non-metastatic castration resistant disease. The clinical course of these patients is extremely variable. Factors that could account for the outcome in the castration resistant M0 patients include: initial criteria for initiation of ADT (PSADT, Gleason's score, time from local treatment to evidence of biochemical recurrence), response to the initial hormonal therapy, PSADT at recurrence, and PSA level in the castrate state [5].

Less than 5 % of patients diagnosed with prostate cancer today demonstrate clinical evidence of distant metastasis at presentation. The survival of men with newly diagnosed metastatic prostate cancer has changed significantly during the past two decades and this is illustrated by the differences in outcome of patients enrolled onto clinical trials over the past two decades. From 1985 to 1986 (pre PSA era) the NCI sponsored an intergroup study comparing leuprolide acetate with or without flutamide in patients with newly diagnosed, hormone naïve prostate cancer. The median survival in the combination arm (best arm) was 31 months. From 1989 to 1993 the Southwest Oncology Group (SWOG) conducted a trial in 1,387 men with newly diagnosed prostate cancer treated with bilateral orchiectomy with or without the antiandrogen flutamide (SWOG 8894, double-blinded placebo-controlled trial) which resulted in no significant differences between arms and the overall median survival in these patients was 33 months [4]. From 1995 to 2009 the SWOG conducted a study in the same patient population (SWOG 9936) which employed in one the arms a GnRH analogue with bicalutamide, median survival data on patients treated with continuous ADT was 49 months [6]. The risk of death observed in SWOG 9936 was significantly lower compared to SWOG 8894 (HR 0.77–0.6–0.8–$p<0.0001$) suggesting a 30 % reduction of risk of death [6] (Fig. 1.3). The proportion of men that present with baseline unfavorable prognostic factors (extent of disease, presence/absence of pain and baseline PSA value) on is significantly lower in SWOG 9936 compared to the SWOG 8894 [6].

The m CRPC survival figures have also evolved in a similar fashion based on clinical trials data. The median survival of patients entered onto the two randomized studies completed most recently in patients with mCRPC in the "pre-chemotherapy

space" is in excess of 30 months. This compares to approximately 19 months in patients with "hormone-refractory disease" reported 10 years ago in the TAX-327 docetaxel study and less than 12 months in the phase 3 mitoxantrone studies conducted in the 1990s.

An important chapter of this book involves a careful description of the extensive research around newer imaging modalities which will undoubtedly enhance our ability to diagnose metastasis at an earlier stage and define the tumor burden more adequately than conventional modalities. In Chap. 4 Cho, Dianat, and Macura describe newer modalities of imaging which may provide new opportunities for treatment besides diagnostics. The authors point out that while earlier diagnosis and treatment of metastasis has not necessarily been proven to extend survival it is likely that identification of patients with early metastasis offers an excellent opportunity for studying this subject. Furthermore, new imaging modalities may facilitate the conduct of clinical by introducing new endpoints and allow for a more adequate assessment of metastatic involvement, quantification of the metastatic burden, and possibly introducing new criteria for response based on functional status of metastatic lesions.

The Biology and Treatment

Prostate cancer growth is driven primarily by androgen receptor (*AR*). Increased understanding of the biology of *AR* signaling and downstream *AR* regulated genes provided the opportunity for therapeutic targeting and resulted in the development of newer compounds that have been shown to improve survival of m CRPC patients prior and after chemotherapy and hence prove the hypothesis that *AR* signaling remains an important regulatory factor in the growth of prostate cancer even after adequate gonadal suppression. In Chap. 3, Nelson and Pienta review the current status and future directions of basic research of molecular mechanisms involved in prostate cancer growth. It is expected that an increase in the understanding of the biology of prostate cancer will continue to provide new opportunities for treatment. The molecular heterogeneity of prostate cancer has long been recognized and emphasized in recent "warm autopsy studies." It is becoming increasingly clear that adequate biological characterization of the disease will allow for a more logical selection of patients and facilitate individualized treatment strategies. Correlational studies evaluating molecular biomarkers, clinical outcomes such as response and toxicities are likely to become hallmarks of drug development in this disease.

The stage migration offers an excellent opportunity to evaluate the clinical transition from the non-metastatic to the early metastatic state as a clinical model for drug development and intervention before irreversible lethal processes are established. This is especially attractive for all treatment modalities currently available including *AR* targeting compounds, bone targeted approaches, non-conventional cytotoxics, and immune-based approaches. If applied early these interventions could affect the rate of disease progression and consequently result in more consequential improvements for patients with relapsed disease.

The sections dealing with current treatments illustrate how new approaches evolved from the laboratory to the clinic. The strategy of targeting *AR* is a perfect example for this. Cheng and Montgomery (Chap. 5) provide an excellent update on the biology of *AR* signaling and discuss the logic for targeting key processes. Feldman, Rathkopft, and Morris (Chap. 6) and Siegel and Ryan (Chap. 7) describe the data on two new approaches targeting the *AR* with antagonists and compounds that further decrease androgen synthesis. The clinical development of these compounds evolved rather rapidly and resulted in FDA approval of enzalutamide and abiraterone for patients with mCRPC. The clinical data and future directions are eloquently discussed by the authors. Docetaxel was the first FDA approved cytotoxic shown to improve survival in patients with mCRPC (about a decade prior to the completion of this book) over the primarily symptomatic approach with mitoxantrone + prednisone. Van Soest, Morree, Sternberg, and de Wit (Chap. 8) provide an update on the role of taxanes in mCRPC and current research efforts focusing on improving outcomes with this modality.

In Chap. 9, Barroso-Sousa and Drake provide an extraordinary description of the rapidly growing body of knowledge on the immune biology of cancer with specific application in prostate cancer. The laboratory and clinical data on the dendritic cells targeting, cell-based vaccine FDA approved in 2010 for patients with m CRPC to the bones and minimal or no symptoms associated with their disease is discussed. The authors provide compelling arguments that indicate that immunotherapy is likely to represent a modality of treatment that will further impact on prostate cancer morbidity and mortality.

Skeletal morbidity is a hallmark of prostate cancer as a result of treatment (androgen deprivation) and the disease (bone metastasis). Gartrell and Saad (Chap. 10) discuss the basic and clinical information dealing with the most important morbidity associated with this disease. Compounds targeting the bone such as bisphosphonates and RANK-ligand biology are available for use in clinical practice. The effects of these compounds extend beyond the outcomes related to bone health associated with androgen deprivation. The interaction between osteoblasts and osteoclast with several other molecular pathways involved in the metastatic processes in prostate cancer is relevant to virtually all modalities of treatment currently available and support a potential role for combining bone targeted treatments other therapeutic interventions. Sartor and Lewis (Chap. 11) further expand on the role for bone targeted treatments with a description of the status of radiopharmaceuticals and the recently FDA approved radium-223. A pivotal clinical trial involving patients with m CRPC, the alpha particles emitter radium-223 combined with conventional palliative care approaches was associated with a significant prolongation of survival compared to palliative care alone. These data support a role for such compounds in CRPC and vigorous research with this approach is warranted.

Chapters 12–20 are devoted to novel targets and clinical development of new drugs in mCRPC. Some of the most promising approaches are discussed including angiogenesis, cellular stress survival processes, and selected specific pathways such as the PI3K/AKT/mTOR/PARP and C-MET/VEGF and moving beyond the genetics, the evolving role of epigenetics and associated therapeutic opportunities are included in specific chapters.

The challenges of identifying new promising compounds and selecting the best methodology that will provide reliable and reproducible data to justify (or not) further testing of new treatments is a monumental challenge not unique to prostate cancer and are clearly discussed by Omlin, Pezaro and de Bono and Garcia and Dreicer. In the era of molecular medicine, molecular characterization of the disease with identification of gene or gene products involved in each patient's disease is clearly becoming a necessity to optimize therapeutic gains. Several examples in other diseases have been described and the strategy of selecting patients based on their molecular genetics has been shown to have merit based on recent FDA approvals of targeted compounds in solid tumors and hematological malignancies. The challenges of obtaining metastatic tissue with biopsies or identifying biomarkers in the blood or circulating tumor cells are discussed and emphasized. Beltran, Kaur, Mossai, Nanus, and Tagawa provide a remarkable update on the group of patients with undifferentiated prostate cancer (Chap. 21). These patients' tumors usually do not express *AR*, are not responsive to ADT, have rapidly growing tumors, frequent visceral involvement, and very short survivals. This subtype of prostate cancer frequently express neuroendocrine features, have unique biological, pathological, and clinical features. Those with the small cell undifferentiated small cell variety are moderately sensitive to radiation and chemotherapy similar to the same type of disease arising in other sites such as the lung. Drs Paller and Smith discuss the important issue of quality of life and patient reported outcomes that remain critical in managing patients with mCRPC (Chap. 22).

Finally, Martin and Oh outline a rational approach to the application of the growing number of therapeutic approaches available in clinical practice today. The recent FDA approval of various new compounds based on pivotal trials

designed to address regulatory criteria of drug approval do not address how new compounds are best used in clinical practice. Prospective randomized trials based on biologic and patient-specific criteria are still needed to shed additional light on the best approach for individual patients.

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Evolution of Clinical States and the Castration Resistant Clinical Paradigm

2

Tian Zhang and Andrew J. Armstrong

Background

Causing more than 233,000 new diagnoses and more than 29,400 deaths in 2014, prostate cancer (PCa) remains a highly lethal disease and accounts for the second most common etiology of cancer-related mortality for men in the USA [1]. Patients with prostate cancer often have disparate clinical courses; while some men undergo active surveillance only and others are cured with local therapies such as surgery and radiation, a portion of men present with metastatic disease or develop an aggressive metastatic disease course despite best local and salvage therapies. The lethal form of metastatic prostate cancer remains a highly heterogeneous disease, however, with prognoses varying from months to many years. Therefore, there is a need to define clinical states of recurrent and resistant prostate cancer and to provide prognostication and prediction around therapeutic decision-making where possible based on the clinical characteristics of prostate cancer progression.

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The discovery of the hormonal dependence of metastatic prostate cancer by Huggins and Hodges in 1941 through the direct clinical observations of symptom relief and biomarker control through orchiectomy and later adrenalectomy led to the initial characterization of the castration resistant disease state [2]. While the biologic underpinnings of progressive disease in the face of castration were not evident until over a half century later, the clinical state of castration resistance has long been appreciated.

Cornerstone work from the Sawyers laboratory demonstrated that reactive up-regulation of the androgen receptor (AR) was prominent in androgen-independent prostate cancer xenografts during serial passaging under castration-like conditions [3]. A variety of mechanisms of resistance to androgen deprivation therapy [4] account for the persistence and escape of disease during low androgen level exposure, including persistent activation of the AR through mutation, amplification, altered co-activators and co-repressors, and c-terminal splice variants, as well as the acquired ability to synthesize or use androgenic precursors [5–9]. These mechanisms show that castration resistant prostate cancer (CRPC) often continues to be dependent on AR for cancer progression at least initially [10], and led to the term CRPC as a replacement for hormone-refractory prostate cancer, given continued dependence on hormonal signaling and the clear activity of AR targeted agents in this disease state. In addition, clinical samples of prostate cancer tissue have demonstrated the persistence of higher androgen levels

F. Saad and M.A. Eisenberger (eds.), *Management of Castration Resistant Prostate Cancer*, Current Clinical Urology, DOI 10.1007/978-1-4939-1176-9_2,

in tissue than serum, sufficient to activate the AR; these tissue androgen levels are likely enhanced through the autocrine and paracrine up-regulation of steroid synthetic enzymes by prostate cancer, mimicking the endocrine adrenal organ in order to survive the castrate state [11, 12]. The term CRPC emphasizes the resistance of the prostate cancer to castration treatment (typically resulting in a serum testosterone level <50 ng/dL), and does not focus on dependence on AR signaling or presence of novel androgen synthesis. Thus, especially in light of new agents such as enzalutamide targeting AR itself as well as abiraterone acetate targeting androgen synthesis (both effective in advanced PCa), CRPC is the more biologically correct term and is now widely used. However, given that these agents do not cure the disease and resistance emerges, redefining prostate cancer disease states based on biologic mechanisms of progression is more desirable and may lead to more rational treatment selection algorithms.

In 2008, the Prostate Cancer Clinical Trials Working Group (PCWG2) defined five categories of clinical CRPC disease states, based on patterns of presentation and dissemination, including (1) clinically localized CRPC, (2) rising prostate specific antigen (PSA) only CRPC, (3) clinical metastases: bone (with or without nodal progression), (4) clinical metastases: lymph node-only CRPC, and (5) clinical metastases: visceral spread with CRPC [13]. At the time, the only systemic therapeutic agents in the pharmaceutical armamentarium against CRPC were docetaxel and mitoxantrone, and clinicians were much more limited in their ability to treat prostate cancer. In 2013, the range of systemic therapeutic agents is much wider, with many more FDA-approved options including immunotherapy such as sipuleucel-T, agents targeting androgen receptor such as enzalutamide, inhibitors of androgen synthesis such as abiraterone acetate, second line chemotherapy agents such as cabazitaxel, and bone-modifying agents such as radium-223, zoledronic acid, and denosumab. With the advent of newer systemic agents, and an improved understanding of the biologic underpinnings of CRPC progression, the clinical course of prostate cancer has changed significantly to warrant a new classification system of the clinical progression of CRPC.

With the breadth of therapeutic agents now available for treatment of prostate cancer, the landscape of CRPC is rapidly changing, and it is important to define the clinical states that patients pass through as they develop castration-resistant disease. Updating the previous clinical states model is needed given the (1) improved understanding of the biology of CRPC, (2) newly approved active agents now commonly used prior to and often instead of chemotherapy, (3) the importance of symptoms, histology, and patterns of metastatic spread in determining treatment sequencing and approval indications, and (4) the improved development of multiple prognostic markers that can better risk-stratify men with CRPC. In this chapter, we will highlight the clinical states of CRPC based on prior exposure to therapy, sites of metastasis, histology, serum biomarkers, and clinical symptoms (Table 2.1). We will end with suggestions for a molecular taxonomy of CRPC that will require clinical context and associations with these clinical phenotypes.

Prior Therapy Exposure

The current clinical states of CRPC remain defined by patterns of spread, but are now heavily dependent on prior therapy exposure (Fig. 2.1), an issue that was not emphasized in PCWG2 given the lack of approved therapies in 2008. For purposes of this book chapter, we will not discuss localized disease, rising PSA after local therapy (biochemical recurrence), or metastatic disease in hormone-naïve PCa. Instead, we will focus on sites of metastatic spread in CRPC and prior therapy exposure. In addition, within each CRPC clinical state, we will discuss the importance of independent prognostic factors including histologic subtype, serum and blood-based prognostic biomarkers, symptoms, and molecular alterations linked to CRPC progression.

As more systemic treatments become available for men with CRPC, it is important to keep in mind prior therapy exposure for each patient. Prior

Table 2.1 Clinical phenotypes of CRPC with implication in prognosis and importance in clinical care

Clinical phenotype	Implications
Pattern of spread	Non-metastatic biochemical recurrence with options for observation, salvage radiation therapy, or hormone therapy Prognostic for survival In order of worsening survival: lymph node-only metastases > bone metastases > visceral metastases (lung > liver)
Gleason grading	Prognostic for overall survival Possibly predictive for sensitivity to docetaxel treatment
Neuroendocrine histology	Prognostic for poor overall survival Lacks sensitivity to hormonal therapy Correlated to Aurora Kinase A and n-myc amplification May be predictive for Aurora Kinase A inhibition
Pain	Prognostic for survival May be predictive of lack of benefit to sipuleucel-T
Anemia	Prognostic for survival
Performance status	Prognostic for survival
PSA levels	Prognostic for survival Changes can be indicative of improvements in survival post-treatment PSA kinetics are prognostic High levels may be predictive of benefit to hormonal therapies and treatment response (AR pathway) Low PSA despite mCRPC may indicate neuroendocrine prostate cancer and lack of benefit to hormonal agents Lower PSA levels may be predictive of benefit with immunotherapy (i.e., sipuleucel-T)
Alkaline phosphatase	Prognostic for survival prior to and during therapy May be predictive of response to ^{223}Ra treatment
LDH	Prognostic for survival Elevated in neuroendocrine prostate cancer
Circulating tumor cell enumeration	Prognostic for survival Post-treatment CTC declines are prognostic with a range of therapies Under evaluation as a surrogate biomarker CTC biomarkers may provide predictive information linked to specific therapies

therapy will dictate prognosis and response to the next treatment strategy, given that cross-resistance to at least several newly approved agents is expected and has been observed in the clinic. The promotion of resistance to subsequent therapy with exposure to novel hormonal agents may be a result of clonal selection, tumor evolution through mutation, or epigenetically regulated cellular plasticity and adaptation. Thus, understanding the prior exposure of a patient to a range of systemic therapies will facilitate the rational sequencing of therapies in the clinic and anticipated clinical benefit from a further line of treatment.

Both of the novel therapeutic agents abiraterone acetate and enzalutamide were tested initially in the post-chemotherapy state, after patients had progressed on docetaxel. However, it is now clear that these agents are likely more active in the pre-docetaxel mCRPC setting [14, 15], based on greater magnitudes and durability of PSA decline and disease control. For example, the PSA response rate and progression free survival for abiraterone acetate in the post-docetaxel CRPC setting is about 50 % and 8 months, while in the pre-docetaxel it is 70 % and 16 months, respectively. In the phase I–II trial of enzalutamide, 65 patients were chemotherapy-naïve and had a better PSA progression free survival [PFS] (greater than 25 % increase in PSA from baseline) compared to the 75 patients who were post-chemotherapy (median PFS not reached for pre-chemotherapy group vs. 27 weeks for post-chemotherapy group) [15]. The reasons for this may relate to lesser disease burden in the pre-docetaxel mCRPC disease state, but may also relate to cross-resistance of hormonal therapy

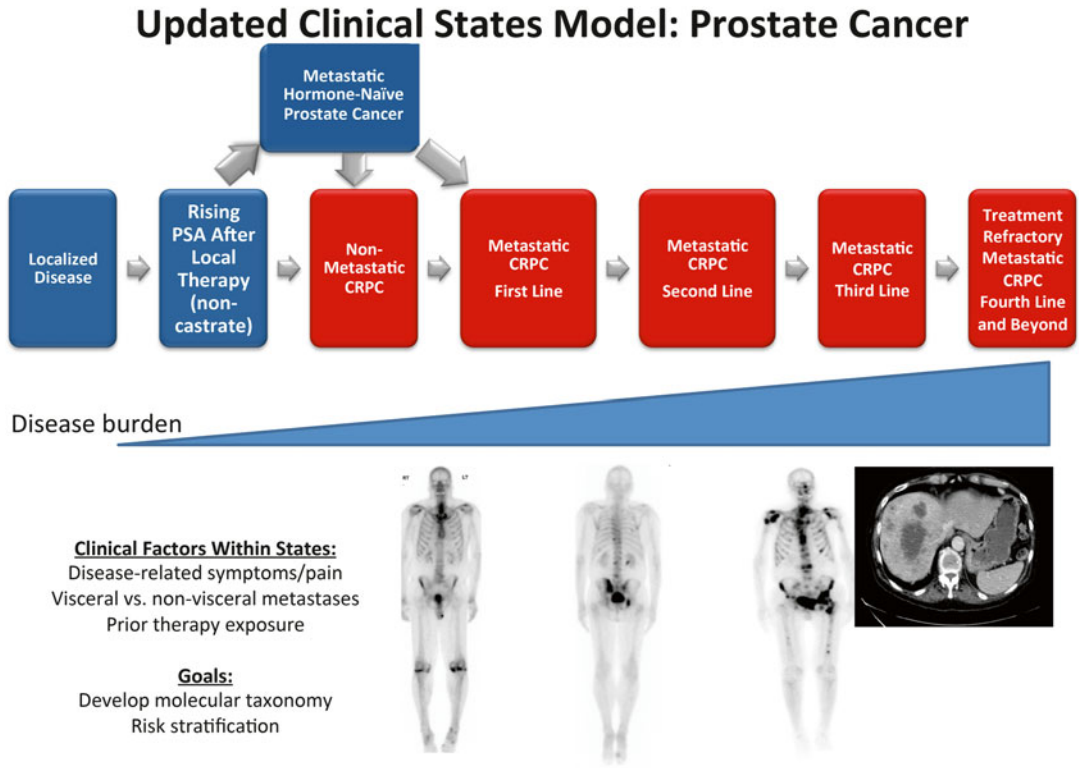

Fig. 2.1 Depiction of evolution of clinical states in prostate cancer

and docetaxel. Recent studies suggest that docetaxel may have an underlying hormonal mechanism, through disruption of AR nuclear transport on microtubule shuttles. Thus, a biologic rationale has emerged that may explain clinical cross-resistance with docetaxel and hormonal therapies. Thus, appreciating prior therapy exposure is essential in predicting clinical benefit to subsequent therapy.

The phase I trial of abiraterone acetate included 19 patients who had prior ketoconazole exposure, and PSA declines of more than 50 % were noted in 9 of these 19 patients (47 %) [16]. Therefore, it appeared as though abiraterone had disease activity in CRPC even in patients who had received ketoconazole previously. Many of these men were not truly ketoconazole-resistant, and thus, it is not clear how active abiraterone would be in truly ketoconazole-resistant men. Studies of enzalutamide have been conducted in men without prior exposure to ketoconazole or abiraterone, and thus it is currently unclear whether cross-resistance exists. However, current retrospective series indicate clear evidence for a lower response rate and duration of response when enzalutamide or abiraterone are used after each other [17–19]. Therefore, the timing and sequencing of treatments can certainly determine disease response to treatment and is essential in determining clinical disease state.

Retrospective studies suggest clinical cross-resistance between enzalutamide and abiraterone acetate. One retrospective study examined 35 patients with CRPC in the post-docetaxel state, who had received abiraterone followed by enzalutamide [19]. In this study, 40 % of patients had a rising PSA as their best response to treatment with primary resistance to enzalutamide. In patients who had some response to enzalutamide (defined by at least one declining PSA value), median time to progression was only 4 months before secondary resistance to enzalutamide developed. Two other retrospective studies examined abiraterone acetate treatment after docetaxel and enzalu-

tamide. One studied 30 patients with CRPC treated with abiraterone acetate after enzalutamide [17] and found that patients had a median time to progression of 15.4 weeks. Only three of the patients had a PSA response with abiraterone. The other retrospective study looked at 38 patients with CRPC who were treated with abiraterone after progression with both docetaxel and enzalutamide [18]. Only ten of these patients (26 %) had any PSA response, and median PFS was only 2.7 months. These data suggest that prior therapy with either abiraterone acetate or enzalutamide may promote cross-resistance with the other agent. While the mechanisms for this cross-resistance are speculative, they may involve the development of AR mutations or ligand binding domain deletion variants that become ligand independent and do not respond to either treatment.

Given the novelty of the systemic therapeutic agents, insufficient prospective data have been generated regarding cross-resistance and underlying mechanisms; biomarkers prospectively defining these mechanisms in the clinic are currently lacking. Until biomarkers of AR and androgen activity are available from clinical specimens, our current disease states remain defined by prior therapy exposure and responses.

Patterns of Spread of Metastatic CRPC

M0 Disease

The site of CRPC metastasis and the presence or absence of metastases is independently prognostic of survival. The majority of men with mCRPC progression through the M0 CRPC disease state, while only a minority of men in current US practice present with metastatic disease (3–5 %) and thus progress to mCRPC without an M0 state [1]. Non-metastatic CRPC is relatively common, with a defined, more indolent, natural history than those with metastatic CRPC [20, 21]. The M0 disease phenotype space is typically asymptomatic and prognosis is dictated by the degree of PSA elevation and the rapidity of PSA velocity or PSA doubling time (PSADT). These two factors can reliably predict the onset and timing of metastatic disease development. For example, time to bone metastases can vary by 6–12 months in men with a rapid PSADT (<4 months) vs. slower PSADT of 6–10 months [22]. Many men with M0 CRPC have an even slower rate of disease progression, with a large minority of men having no progression to metastases within 2–3 years [22]. The goal of treatment in patients with M0 disease is to prevent or delay symptomatic metastases while also increasing OS without undue toxicity. Non-metastatic CRPC or locally advanced CRPC is therefore a clear clinical state of CRPC with distinct outcomes [13]. Currently, several trials of active systemic hormonal agents (enzalutamide, ARN-509) are in phase III trials evaluating the role and clinical benefit of delaying metastasis in this setting, as opposed to the current indication of waiting for mCRPC to develop before utilizing these agents.

Recently, a trial of denosumab, a monoclonal antibody to RANK ligand (RANKL), studied time to skeletal metastasis in patients with CRPC without metastasis but with either high PSA > 8 μg/L or PSA doubling time <10 months [23]. Denosumab treatment delayed time to first skeletal metastasis from 29.5 months to 33.2 months (HR 0.84, $p = 0.032$) in the placebo group and significantly increased bone-metastasis-free survival from 25.2 to 29.5 months (HR 0.85, $p = 0.028$). However, OS was not significantly different between the two groups, the differences in time to bone metastases were not felt to be clinically meaningful, and thus denosumab has not approved for use prior to radiographically apparent metastasis. This trial, however, provides a strong dataset to study the natural history of M0 CRPC and frame future clinical trials of more active agents.

As more novel therapeutics are approved in the metastatic and post-chemotherapy setting, they are being evaluated as first-line therapy prior to docetaxel given their favorable toxicity and efficacy. For example, enzalutamide is currently being tested in metastatic CRPC patients in the pre-docetaxel setting, as well as in non-metastatic patients in the multicenter, placebo-controlled,

double blind Phase 3 PROSPER trial. Thus, the M0 CRPC disease state is emerging as an important state, given the desire of patients and providers to delay as long as possible the onset of symptomatic metastatic disease.

Lymph Node Metastases

PCa metastasizes to lymph nodes in up to 10 % of patients at the time of diagnosis [24]. Men with node positive disease at the time of surgery have poorer prognosis compared to men without nodal metastases, but still have a median survival in excess of 11 years in the absence of any immediate therapy, and over 13 years with immediate hormonal therapy [25]. Of 3463 PCa patients in a retrospective analysis at the Mayo Clinic, 322 had lymph node metastasis. These patients had a 10-year PFS of 64 % with 10-year cancer-specific survival rate of 83 % [24]. A separate retrospective study in 2013 examined 369 men at Memorial Sloan Kettering Cancer Center with lymph node metastases and found their 10-year cancer-specific survival rate to be 72 % [26].

Men with lymph node-only metastasis and CRPC have a longer PFS and disease-specific survival when compared to those with bone or visceral metastasis [27]. The TAX-327 study prospectively randomized 1,006 men with mCRPC to receive either docetaxel or mitoxantrone, and OS prognosis depended on the site of metastatic spread [27] (Fig. 2.2). Patients who had lymph node-only CRPC had the best median OS of 35 months. In comparison, men with bone CRPC had median OS of 19.5 months, and patients with visceral disease had the lowest median OS of 14.5 months ($p<0.0001$) [27]. Thus, lymph node-only prostate cancer and lymph node-only CRPC have relatively favorable prognosis, and clearly, the site of metastatic spread has independent prognostic significance.

Bone Metastases

Bone metastases are found in over 95 % of men with lethal CRPC [28]. The reason for poorer OS in patients with bone metastases in CRPC as compared with node-only mCRPC may lie in the different mechanisms of hematogenous vs. lymphatic dissemination of tumor cells, the formation of sclerotic bone metastases that are highly resistant to therapies, or the greater disease burden in these men. This mechanism may depend on PCa cells undergoing a phenotypic change to a more mesenchymal or osteomimetic state, developing the ability to invade blood vessels, and colonizing the bone marrow by extravasating out of the blood vessel. This process, termed epithelial-to-mesenchymal transition (EMT), may lead to the acquisition in prostate cancer of

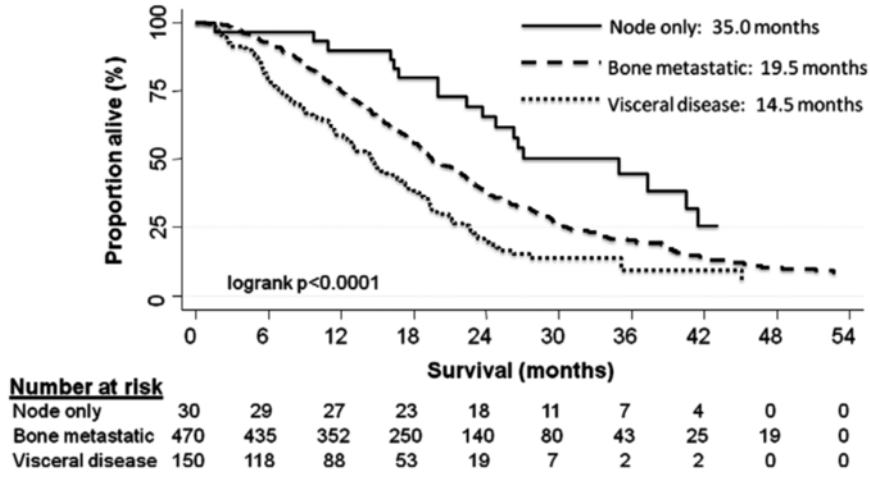

Fig. 2.2 Kaplan–Meier overall survival estimates for patients in the TAX-327 trial as separated by node-only disease, bone metastases, and visceral disease [27]. Reprinted from [27], with permission from Elsevier

stemness properties that permit homing of the cancer to bone, and has been observed in disseminated tumor cells and circulating tumor cells (CTCs) of men with mCRPC and in preclinical models of PCa [29–33]. Chemokines, inflammation, bone stroma, circulating mesenchymal and stem-like progenitor cells, and other host factors may regulate this process, and intrinsic oncogenic programs to the PCa cell may promote bone metastases [29, 34–36]. Once the PCa cells are situated within the bone marrow niche, they interact with signaling molecules such as transforming growth factor beta (TGFβ), vascular endothelial growth factor (VEGF), and RANK ligand (RANKL) to acquire a bone-like primitive phenotype [29], compete for the niche of hematopoietic stem cells and lead to progressive bone marrow failure [29, 37]. This plasticity or dual epithelial–mesenchymal nature of CRPC has been observed in bone metastases and CTCs from men with CRPC, indicating the importance of molecular pathways regulating stemness, differentiation, and plasticity during the development of bone metastasis [30, 33, 38]. The clinical significance of this bone-homing program is obvious once bone metastases have developed; however, there is a lack of validated biomarkers in localized prostate cancer that can predict for the onset of bone metastases.

Patients with CRPC who develop bone metastases can derive benefit from therapy that targets the bone microenvironment, including bisphosphonates such as zoledronic acid [39], RANKL inhibitors such as denosumab [40], and the radiopharmaceutical radium-223 [41]. The denosumab trial randomized 1,904 patients with CRPC metastatic to bone to treatment arms of either denosumab or zoledronic acid. The time to first skeletal event was the primary endpoint. Patients treated with denosumab had median time to the first skeletal event of 20.7 months, an improvement of 3.6 months when compared to 17.1 months for those treated with zoledronic acid (hazard ratio 0.82, $p=0.0002$) [40].

A novel radioisotope, radium-223 chloride, was recently approved by the FDA to treat PCa bone metastases. The ALSYMPCA phase III trial enrolled 921 patients who had only bone metastases and were either post-docetaxel or pre-docetaxel due to medical fitness or patient refusal [42]. These patients were given radium-223 (50 kBq/kg intravenously every 4 weeks) for 6 treatments or placebo. Patients on the radium-223 arm had a higher median OS of 14.9 months compared to 11.3 months in patients treated with placebo ($p<0.001$) and demonstrated longer time to first skeletal event at 15.6 months compared to 9.8 months, respectively ($p<0.001$) [42]. Thus, bone metastases are a clinical disease state that determines treatment decision-making and the use of specific bone-targeting agents that are not known to exhibit activity outside of the bone. Future work to define biomarkers that can reliably predict for the development of bone metastases are needed in order to develop trials of systemic agents for the prevention of bone metastases.

Visceral Organ Metastases

When CRPC metastasizes to visceral organs such as lungs and liver, the disease course is often more aggressive than CRPC with metastasis to lymph nodes and bones (Fig. 2.2). Men with lung or liver metastases appear to benefit from both novel agents such as enzalutamide and abiraterone acetate, as well as traditional chemotherapy agents such as docetaxel. However, patients have much lower rates of response and survival once visceral metastases, particularly liver, occur. Patients with visceral metastases are currently restricted from receiving certain systemic therapies, such as radium-223, per the FDA label. In addition, current NCCN guidelines do not recommend the use of sipuleucel-T in men with liver metastases, given that these men have a poorer prognosis, and men with visceral metastases were excluded in the phase III trial of sipuleucel-T.

Data from the TAX-327 trial demonstrated that men with metastatic CRPC to the liver had the poorest prognosis (10 months), as compared to men with lung metastases (14.4 months) [43]. This observation is reflected in published nomograms in this population, with strong negative impact on survival in the presence of liver metastases [44, 45]. A recent poster presentation at the 2013

ASCO annual meeting also showed that patients with metastatic CRPC to lungs alone had a median OS of 15.5 months, compared to 7.7 months in those patients who had metastatic CRPC to liver or liver+lung [46]. Thus, the pattern of visceral spread is also an important consideration in determining clinical phenotype in mCRPC.

Histological Categories of CRPC

The histology of PCa is commonly based on their origin from glandular-forming epithelial cells. While the cell of origin of PC is debated and may be the basal cell of the prostate, a subset of a luminal cell population, or both depending on context, typical prostate cancer is adenocarcinoma and consists of glandular architecture with varying degrees of loss of differentiation. Cells in the normal male prostate do not normally proliferate, but are able to survive repeat bouts of castration and regeneration. However, over time, with cumulative mutations and epigenetic lesions, PCa cells acquire the ability to continuously proliferate despite AR ablation, and acquire an invasive phenotype [3]. The Gleason grading system was initially described in 1966 [47], and was validated as a prognostic measure at the Minneapolis Veterans Administration in 1974 [48]. This grading system was updated in 2005 to correspond more closely with patient outcome [49]. As a score of nuclear polymorphism, glandular disruption, disease heterogeneity, basement membrane disruption, and de-differentiation of prostate cancer, the Gleason score reflects the aggressiveness of the individual PCa. The Gleason sum continues to be independently prognostic for survival in the CRPC setting, as demonstrated repeatedly in multiple nomograms for prostate cancer survival [44, 50]. The Gleason sum has also been found recently to be potentially predictive for treatment response and sensitivity to treatment with docetaxel in the TAX-327 trial [51]. In this analysis, higher tumor grades (Gleason score $\geq$7) had a more pronounced survival benefit from treatment with docetaxel than with mitoxantrone [51]. Thus, higher Gleason grading can be both prognostic for survival and potentially predictive for a greater magnitude of treatment benefit from docetaxel. While this requires confirmation, tumor grade may provide a surrogate biomarker for genomic instability, proliferation, and de-differentiation that may be exploitable in the clinic and provides independent prognostic information.

Adenocarcinoma is the main histologic form of PCa, accounting for 95 % of all PCa [52]. The remaining 5 % is made up of ductal, mucinous, small cell, and anaplastic carcinoma, each of which comprises a more aggressive variant that may progress to CRPC more rapidly and behave as high grade tumors. In the national SEER database, there is an incidence of 61 cases per 10,000 people per year for mucinous carcinoma, 49 cases per 10,000 people per year for ductal carcinoma, and 35 cases per 10,000 people for anaplastic or neuroendocrine carcinoma [53]. Mucinous carcinoma has similar 5-year OS to adenocarcinoma (75.1 vs. 76.5 % respectively), and ductal carcinoma has a slightly more aggressive disease course, with a 5-year OS rate of 61.7 % [53].

Contrastingly, neuroendocrine prostate cancer (NEPC) is much more aggressive and has a 5-year OS rate of only 12.6 % [53]. NEPC can arise either de novo with a low serum PSA level, obstructive symptoms, and often distant metastases at the time of diagnosis, or more commonly, NEPC emerges as a secondarily resistant subtype after prostate adenocarcinoma treatment. NEPC is independent of AR signaling and can be a mechanism of castration resistance (Table 2.2). NEPC often overexpresses chromogranin A and synaptophysin, biomarkers which are detectable in serum [54]. Many molecular alterations accumulate in NEPC, including amplification of Aurora Kinase A and N-myc [55], overexpression of EZH2 [55], loss of Rb [56], and activation of the PI3 kinase pathway [57]; these represent potential therapeutic targets. During therapy with an Aurora Kinase A inhibitor, these tumors revert to adenocarcinoma features, indicating histologic plasticity [55]. This phenomenon mimics what is observed in the reversible transitions between small cell and non-small cell lung carcinomas [58]. This toggle effect raises the possibility that prostate cancer cells have an inherent plasticity to change histological subtypes when evading treatment pressures and imply that the current or

Table 2.2 Prostate cancer categories based on androgen and AR dependency

Categories of prostate cancer based on androgen ligand and androgen receptor activity	Examples
1. Androgenic ligand dependent, androgen receptor dependent	Wild-type AR, AR mutants, AR amplification, autonomous androgen synthesis
2. Androgenic ligand dependent, androgen receptor co-opted	Glucocorticoid receptor, other promiscuous ligands
3. Androgen independent, androgen receptor dependent	AR variants, AR deletions, non-canonical AR activation
4. Androgenic ligand independent, androgen receptor independent	Neuroendocrine prostate cancer, basal/stem-like prostate cancer, EMT?

prior histologic appearance of a CRPC patient's tumor may be important clinically.

NEPC correlates with poor clinical prognosis [53]. A series of 21 patients with NEPC were treated with cisplatin-based chemotherapy active in small cell carcinoma of the lung, with a resulting median OS of 9.4 months (range 1–25 months) [59]. A phase II trial of cytotoxic chemotherapy in 120 neuroendocrine prostate cancer patients subsequently treated patients first with carboplatin and docetaxel (CD), followed by cisplatin and etoposide (EP) [60]. Primary endpoints included response rates and time to progression. Only 74 patients were able to undergo treatment with both regimens. Of these men, 50 % had benefit from both regimens, 34 % responded to CD but not to EP, 9 % responded to EP but not to CD, and 7 % did not respond to either regimen. Median OS was 16 months [60]. Therefore, NEPC carries a poor prognosis despite multi-agent platinum chemotherapy. The emerging biologic differences inherent in NEPC transformation however suggest that molecularly targeted therapies, likely in combination, may have specific activity in this disease. The Aurora Kinase inhibitor MLN-8237 is currently in a phase II trial which is actively enrolling patients with NEPC to evaluate drug efficacy and predictive biomarkers (ClinicalTrials.gov number NCT01799278). Thus, NEPC represents a distinct CRPC histologic and molecularly defined entity. Current barriers, however, include defining the clinical characteristics of men with CRPC who have this NEPC genotype.

Symptom as Indicative of Clinical State of CRPC

Pain

CRPC patients often have pain but can have metastatic disease without the presence of pain. Pain often arises when CRPC metastasizes to bone and is often described as dull and achy, migratory, and sometimes progressing to sharp pain in axial more than appendicular regions of the skeleton. However, many patients develop multiple sites of metastatic disease in the absence of pain. In the TAX-327 trial, half of the patients who had multiple areas of high uptake on bone scan did not have pain, while the other half required opioid medications to control pain (unpublished data). Systemic therapy may also induce clinical response in pain without PSA response [61, 62]. The measurement of pain should be objectively ascertained, using reliable and validated surveys or patient reported outcomes. Cancer-specific pain requiring opioid analgesia is considered significant pain in current prognostic models of CRPC.

Although pain does not correlate with presence of metastases, clinically significant pain is an independent prognostic indicator for OS and is included in current nomograms for survival in CRPC [44, 45]. In TAX-327, patients who experienced pain relief with treatment had a higher median OS of 18.6 months, compared to 12.5 months for patients who did not experience pain relief [61]. Similarly, a large analysis of NCI cooperative group trials of men with CRPC also demonstrated that more severe pain was associated with shortened survival (17.6 vs. 10.2 months for men with low vs. high pain scores [63]). These results have been repeated in studies of abiraterone acetate as well, and therefore novel hormonal agents can also change this clinical manifestation of disease with subsequent improvement in prognosis [64]. Pain and symptomatic CRPC is currently included in the

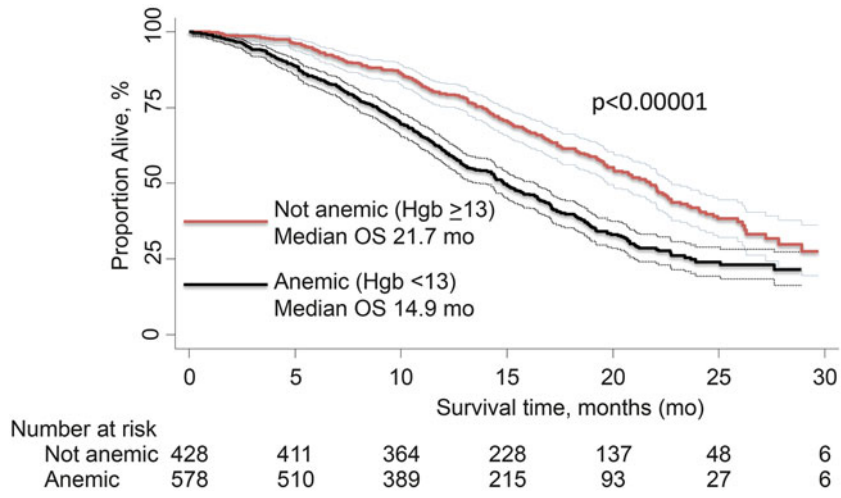

Fig. 2.3 Kaplan–Meier overall survival estimates for patients with mCRPC in the TAX-327 trial with anemia (hemoglobin <13 g/dL) vs. patients without anemia (hemoglobin ≥13 g/dL). Data is based on Armstrong et al. [44]

approved USFDA labels and NCCN guidelines for sipuleucel-T (absence of significant symptoms) and radium-223 (presence of symptoms). Thus, the pain phenotype connotes a meaningful impact on outcomes, quality of life, and treatment selection of a man with CRPC.

Pain relief and prevention is clinically significant. A response in pain led to the FDA approval of mitoxantrone chemotherapy in CRPC [65], and the prevention of skeletal related events led to FDA approvals for zoledronic acid and denosumab [39, 40]. Ongoing phase III trials of the dual VEGFR2/c-MET inhibitor, cabozantinib, in patients with CRPC also have durable pain palliation as an endpoint under evaluation for regulatory approval, provided these improvements are associated with improved survival and are substantial and clinically meaningful. Thus, pain is clinically significant and is prognostic for inferior survival outcomes. Treatment selection may also depend on pain severity and response, as several of the systemic therapies in the treatment sequence may be selected due to their intended effect on clinical pain.

Anemia

Anemia-related fatigue is a complicating symptom in a subset of patients with CRPC [66, 67]. Anemia can be attributed to multiple causes, including anemia of chronic disease, androgen-deprivation therapy, chemotherapy toxicity, renal disease, disseminated intravascular coagulation (DIC), blood loss, or bone marrow infiltration of prostate cancer cells. Bone marrow infiltration results from prostate cancer cells acquiring a stem-like phenotype and taking over the bone marrow niche of hematopoietic stem cells [29]. Anemia can therefore indicate bone marrow infiltration and ultimately bone marrow failure, which contributes directly to patient mortality.

Anemia in men with CRPC is an independent prognostic marker for worse prognosis; it has been shown in multivariate analysis to be an important symptom in all published nomograms for survival in metastatic CRPC [44, 45, 50, 66, 68]. From the TAX-327 database, anemia contributed to a median OS of 14.9 months compared to 21.7 months in patients without anemia (Fig. 2.3).

Although erythropoietin to stimulate red blood cell production has been shown to increase hemoglobin levels and increase quality of life in some patients with CRPC [69, 70], a Cochrane meta-analysis ultimately showed that erythropoietin increases rates of hypertension, thrombocytopenia, and venous thromboembolism in patients with solid tumors, and may accelerate tumor progression in several cancer types [71]. Therefore, erythropoietin is only given if the

severe anemia is caused by chemotherapy, and then only after an informed consent discussion, using a hemoglobin level of less than 10 g/dL as the indication for treatment initiation and continuation of treatment only if the hemoglobin remains less than 10 g/dL. In the circumstance of marrow infiltration by tumor, anemia generally improves with effective PCa therapy, including chemotherapy and hormonal therapies, which may also reverse coagulopathy.

Performance Status

The Karnofsky Performance Status (KPS) has been well established as an important prognostic factor in multiple nomograms and multivariate analyses of survival in men with mCRPC [44, 45, 50, 68]. KPS often depends on patient state at time of presentation. KPS can be affected by comorbidity and prior medical conditions, but also can reflect other symptoms such as pain, anemia, emotional distress and mood changes after a cancer diagnosis. Thus, functional status is of utmost importance both in determining prognosis and in leading the patient and provider to selecting specific systemic therapies. However, KPS remains a crude measure of functional activity in men with asymptomatic mCRPC or M0 CRPC or earlier disease states. Other tests of functional activity, such as exercise tolerance or geriatric functional assessment scales, may provide a greater assessment of physiologic reserve. The ability of novel hormonal agents such as enzalutamide and abiraterone acetate, as well as the radiopharmaceutical radium-223, to improve survival in men with metastatic CRPC and impaired performance status opens up several important treatment options for most men and marks an important milestone in the history of PCa treatment [14, 42, 72]. However, these hormonal agents may also impair functional status in men with asymptomatic CRPC due to loss of lean muscle mass and gain of metabolic derangements and fat mass [73]. Thus, attention to functional status in CRPC is critical in the assessment of treatment selection, response, and toxicity.

Biomarkers Indicative of Clinical States of CRPC

Multiple serum or blood-based biomarkers have prognostic significance in the progression of clinical states of CRPC, including PSA, alkaline phosphatase (AP), lactate dehydrogenase (LDH), albumin, and CTCs [74]. With the exception of CTCs, most of these biomarkers have been shown in nomograms with multivariate analyses to be independently prognostic of patient survival from CRPC as well as have implications for treatment selection, and CTCs have also been independently associated with mortality in men with metastatic CRPC [75]. For example, AR signaling regulates PSA production, and increasing PSA levels may indicate AR pathway dependence, while low PSA levels despite metastatic progression such as in NEPC indicates androgen receptor independent prostate cancer (ARIPC). Patients with intact wild-type AR are dependent on AR signaling and appropriate for AR-targeting therapies; however, those patients who develop splice variants of AR with C-terminal deletions would not benefit from AR-targeting agents. In addition, patients can further develop ARIPC, where cellular proliferation is no longer dependent on AR signaling. In these cases of ARIPC, cytotoxic agents are potentially more beneficial. Bone biomarkers such as AP may reflect the tumor burden within bone and potential benefit with bone-targeted therapies.

Prostate Specific Antigen

PSA was developed to screen and diagnose PCa, as well as to track the response of PCa to therapies. Although PSA is controversial as a screening tool for PCa, PSA is widely considered to be a useful tool to monitor disease recurrence and response to local and systemic therapies. PSA levels and PSA kinetics are independently prognostic for survival across all PCa disease states, including M0 and M1 CRPC [20, 45]. Declines of least 30 or 50 % in PSA during treatment with chemotherapy are highly associated with improvements in OS [61]. For example, in the TAX-327

trial, men who attained PSA reduction at least 30 % during the first 3 months of chemotherapy had a median OS of 21.6 months, compared with 13.0 months for patients who had less than 30 % PSA decline, and this threshold of decline had the greatest association with survival, despite relatively modest surrogacy [61]. A phenomenon that reduces the ability to associate PSA declines with survival outcomes is the transient rises that occur during the initiation of systemic therapy. Transient PSA rises can occur in 15–20 % of men with CRPC in the first 3–4 months of chemotherapy, can be of substantial magnitude (60–400 % rises have been reported), and have usually resolved by cycles 3–4 of chemotherapy [62]. These transient rises in PSA do not confer prognostic significance, and clinicians should continue treatment through isolated changes in PSA during the first few cycles of systemic treatment.

Systemic treatments targeting the AR axis such as standard ADT and anti-androgens, enzalutamide, and abiraterone acetate can all induce responses with PSA declines. However, since PSA and radiographic/clinical changes do not often parallel each other, as well as since PSA can drift upwards for relatively long (3–6 months) durations of time without clinically apparent effects, the decision to discontinue systemic agents should not be made based on PSA changes alone [76]. For certain agents, benefit in survival does not correlate with PSA levels. For example, systemic therapies such as sipuleucel-T and Radium-223 can improve OS without changing PSA levels, while agents such as docetaxel and bevacizumab combinations can evoke larger short-term PSA declines without any survival benefit [77, 78]. Therefore, PSA levels can be informed prognosis and can be tracked over time to update prognosis, and PSA levels themselves may indicate underlying tumor biology. For example, the benefit of sipuleucel-T may be greater in men with a lower tumor burden, as evidenced by the greater survival benefit seen with sipuleucel-T in men with a PSA level prior to therapy of under 20 ng/mL, as compared to the relative benefit with higher levels of PSA [79]. Thus, PSA is a biomarker of androgen-AR axis activity, tumor burden, prognosis, and may be predictive in certain contexts. Changes in PSA as a biomarker of response to systemic therapies in CRPC need to be considered in the context of the mechanism of action of the next line of treatment.

Alkaline Phosphatase

The bone biomarker alkaline phosphatase (AP) is frequently elevated in men with bone metastatic CRPC, and serum AP levels are independently associated with survival from CRPC [44, 50]. In their initial seminal paper on the hormone dependence of prostate cancer, Huggins and Hodges described transient increases in serum AP and declines following orchiectomy [80]. The TAX-327 trial included men with multiple bone metastatic lesions, only 60 % of whom had elevations in AP, indicating that AP release is independent of metastatic disease burden (unpublished data). The TAX-327 trial also demonstrated that elevated AP levels are prognostic of worse OS. Patients with high AP levels above 200 IU/dL had a median OS of 21 months, compared to 14.7 months for patients with lower AP levels below 200 IU/dL (Fig. 2.4). In addition, AP levels may transiently rise and then decrease with effective systemic therapy, such as docetaxel. These declines in AP are also important for prognosis. Normalization of AP with chemotherapy, for example, was associated with a meaningful 20 % relative improvement in survival, independent of PSA reductions [81]. These data indicate that AP levels report on an important disease phenotype and state in CRPC with important prognostic implications over time.

Bone biomarkers such as AP or osteolytic markers of C- or N-telopeptide levels are both prognostic for survival and change with therapy. These markers may predict benefit from further systemic therapies from both bone-targeted therapies and docetaxel [81, 82]. Elevations or reductions in urinary N-telopeptide may also be associated with the onset of skeletal events including pain requiring radiation or surgery to bone, spinal cord compression, and pathologic fracture [83]. Patients with high bone AP levels derived higher benefit from radium-223 [41].

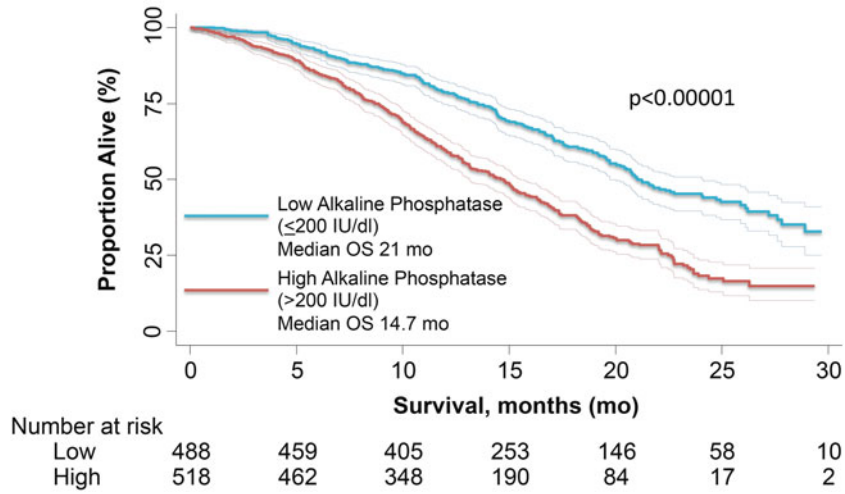

Fig. 2.4 Kaplan–Meier overall survival estimates for patients with mCRPC in the TAX-327 trial with alkaline phosphatase (AP) elevation (>200 IU/dL) vs. patients with normal AP (≤200 IU/dL). Data is based on Armstrong et al. [44]

In the phase III trial of radium-223, patients who had baseline AP >220 U/L treated with radium-223 had a longer median OS compared to those treated with placebo (HR 0.62, 95 % CI 0.49–0.79, $p<0.001$), while those with normal AP had no significant survival benefit [42]. This study showed that high AP levels may predict for benefit in OS from the bone-targeting agent radium-223 [42], but prospective trials are needed to study the exact potential mechanism and thresholds for predictive value. Similarly, treatment responses with lower AP levels after radium-223 have been shown to have prognostic significance for improved survival [84]. Similar changes in bone-derived biomarkers are highly associated with outcomes in both zoledronic acid and denosumab [82]. Thus, AP and bone biomarker elevations are important clinical characteristics that report on the biology of bone metastases in patients with CRPC and need to be kept in mind when choosing systemic therapies, particularly those that have direct impact on the bone microenvironment. Currently, however, the use of these bone-targeted agents is not restricted to men with bone metastatic based on bone biomarker levels. Clinicians need a prospective, controlled evaluation of these bone biomarkers as a tool to guide the initiation or discontinuation of bone-modulating therapeutic agents.

Lactate Dehydrogenase

Lactate dehydrogenase (LDH), an enzyme active in glucose metabolism, is often elevated in tumors which undergo high rates of cellular metabolism and proliferation, but may also reflect cellular turnover or hypoxia/necrosis. LDH is not a tissue-specific biomarker, and elevations can be seen with red cell diseases, muscle diseases, and myocardial infarction. High LDH levels are common for patients with CRPC and independently prognostic for poor survival; thus, high LDH levels are indicative of disease aggressiveness [50]. In fact, high LDH is one of the worst prognostic discriminators in CRPC, on a similar order as visceral metastases and pain, reflecting the importance of measuring this biomarker in clinical trials and in the clinic. This is similar to the independent association of elevated LDH levels with death in a number of other malignancies including breast and kidney cancer, melanoma, and lymphoma. Similarly, in patients with NEPC, high LDH levels and low albumin levels have correlated with poorer disease-specific survival. A series of patients with NEPC at MD Anderson showed that patients with high LDH and low albumin levels had a median disease-specific survival of 4.1 months, compared to 13.1 months for all patients [85]. Thus, serum LDH elevation is

highly prognostic, with an aggressive disease course, and should be taken into consideration when selecting the next line of treatment. While LDH is not predictive of the benefit of a specific therapy, recent studies of LDH levels as a predictive biomarker in kidney cancer for PI3K/mTOR-based therapeutics raise the possibility that LDH may be a biomarker of oncogenic pathway activity and could help select for molecularly targeted agents. Thus, LDH is an important longitudinal prognostic biomarker and defines a particularly aggressive CRPC disease state.

Chromogranin A and Synaptophysin

NEPC also occasionally secrete neuropeptides such as chromogranin A (CgA), neuron-specific enolase (NSE), and synaptophysin, all of which become elevated in serum and tissue. Other anaplastic variants of CRPC may produce carcinoembryonic antigen (CEA). These biomarkers can be monitored during the course of treatment for patients with NEPC or anaplastic variants of CRPC [60]. Elevated serum CgA levels were found to be independently prognostic for worse survival outcomes in men with CRPC [86]; however, it is yet unknown what specific level of CgA elevation is enough to indicate NEPC. In addition, it is also unknown whether CgA elevations can be used to predict benefit from alternative systemic therapies, such as platinum-based chemotherapies. Thus, serum neuroendocrine markers can be useful for prognosis and monitoring of individual NEPC or anaplastic variant patients during treatment, but more prospective studies of these biomarkers for treatment decision-making need to be performed.

Circulating Tumor Cell Enumeration

In addition to the above serum biomarkers, enumeration of CTCs with the CellSearch® assay is also independently prognostic in men with mCRPC [75]. The assay uses magnetic beads to antibodies against epithelial cell adhesion molecule (EpCAM) to capture tumor cells in peripheral blood. The captured cells are differentiated from white blood cells using exclusion of cells that express CD45, and further manually counted. As defined by this method, a CTC is nucleated, greater than 4 μm, and cytokeratin positive, CD45 negative. Using the CellSearch® assay, CTCs are frequently present in men with metastatic CRPC. Patients with CRPC with ≥5 CTCs per 7.5 ml of blood had decreased OS of 11.5 months when compared to 21.7 months in patients who had <5 CTCs per 7.5 ml of blood, $p < 0.0001$ [75]. In addition, CTC enumeration can change prior to PSA declines during the course of treatment and may be more sensitive in assessing treatment response [75]. Declines in CTCs after treatment are also highly prognostic, and prognosis can be updated based on CTC enumeration. However, many men have radiographic and clinical progression of CRPC in the absence of CTCs by the traditional Cellsearch® method, suggesting that current methods of detecting CTCs are insufficient and newer methods need to be developed. For example, only about 40 % of men with progressive mCRPC have >5 CTCs per 7.5 mL of whole blood, and CTCs are frequently undetected in men with asymptomatic CRPC, despite ongoing progression and metastatic dissemination.

In subsequent analyses of the Cellsearch® assay and captured CTCs, patients seem to have a variable expression of EpCAM-positive CTCs. EpCAM expression can be lost and therefore CTCs can escape the current technologies for CTC capture; this phenomenon has been demonstrated in both patients with metastatic disease to the brain and patients with triple negative breast cancer [87–89]. In addition, patients with visceral metastases and mCRPC tend to have lower than expected numbers of CTCs but poor overall prognosis [90]. Patients with mCRPC commonly co-express epithelial and mesenchymal/stem cell markers on the CTC, indicating epithelial plasticity and suggesting that other cell surface markers may be useful to detect mesenchymal CTC which have lost expression of EpCAM [30]. CTC levels in men with CRPC are also independently associated with survival and may not be linked with AP,

PSA levels, or pain, indicating that this clinical presentation of CTC elevation cannot be predicted based on other clinical features [90, 91]. Changes in CTC phenotype based on PSA expression or PSMA expression also illustrate the emerging heterogeneity of CRPC progression [92]. Further research remains to be performed to better understand and characterize CTCs and to enable more sensitive detection of CTCs. The real potential of CTC research lies in the ability to utilize CTC biomarkers as a noninvasive detection of tumor biology in real time, thus linking direct predictive biomarkers with specific therapies. Whole genome RNA and DNA methods to characterize CTCs molecularly, including such oncogenic lesions as AR status and PTEN loss among other molecular alterations are being examined to discover further targets for therapy and to understand mechanisms of drug resistance in CRPC. These studies will serve to better define the molecular taxonomy of CRPC and allow the merging of clinical phenotype and outcomes research with genotype.

Molecular Changes in CRPC

Although there is clear connection between clinical characteristics of CRPC to survival, molecular alterations in CRPC have not been clearly linked to clinical disease course or prognosis. Unlike the step-wise progression of clinical states of CRPC, it is unclear which molecular lesions occur early during disease progression and which lesions occur later. However, many molecular alterations have been characterized in CRPC. These include clonal fusions in the ETS-family of transcription factors (i.e., TMPRRS2-ERG) [93, 94], heterogeneous loss of tumor suppressors such as PTEN [95], p53, and Rb [56], as well as activation of the PI3K [57] and Ras pathways, in addition to amplification of AR and c-myc [96–98]. The common loss or gain of epigenetic regulators or transcription factors such as EZH2, CHD1, SPOP, SPINK, and others illustrates the need to identify these lesions in men with CRPC and develop associations of these molecular aberrations with responses to treatment and survival.

Whole genome analysis performed in seven prostate cancers recently identified numerous chromosome rearrangements and gene fusions, with a median of 90 rearrangements per cancer genome [93]. Gene rearrangements of the Ets transcription factor family (especially *ERG*) fused with a partner, usually regulated by the androgen receptor (especially *TMPRSS2*) [94] are the most common genomic changes. The clinical disease course of the *TMPRSS2:ERG* fusion is not well understood, particularly once CRPC has developed, with mixed results in several retrospective patient panels after prostatectomy, using the primary outcome of biochemical recurrence [99–101]. Fusion appears to promote early cancer initiation and invasion and therefore, in metastatic CRPC, the presence of these fusions does not necessarily play a role in determining outcomes.

Another genomic analysis of 57 prostate cancers showed that genetic alterations evolved in a coordinated fashion, which was termed chromoplexy [98]. Through genomic analysis of CRPC, AR signaling is often found to be altered, including point mutations in the *AR* gene, *AR* gene amplification, or splice variants of AR [96, 97, 102]. Several groups identified multiple mutations in AR cofactors, including MLL2, FOXA1, UTX, and ASXL1-3 [93, 103, 104]. SPINK1, CDK12, Ras/Raf, and SPOP mutations have also been implicated in many prostate tumors [93, 103, 104], indicating distinct genotypes in PCa that lack ETS family fusions. The focus of future research should link these genotypes to clinical phenotypes, therapeutic interventions, and clinical outcomes.

Several other targetable lesions are also found in CRPC (Table 2.3). These include loss of the tumor suppressor genes such as *PTEN* [95], *RB1*, or *TP53* [98] and activation of the oncogenic PI3K/Akt and Ras pathways [57]. The future challenge remains in associating these molecular aberrations with clinical phenotypes, as well as potential treatment targets such as PI3K inhibitors or cell cycle checkpoint inhibitors (for Rb wild-type patients) in the clinical setting.

As mentioned above, 40 % of NEPC have amplification of *AURKA* and *MYCN*; these amplifications were found in only 5 % of prostate

Table 2.3 Categories of resistance, biologic targets, and potential clinical treatment strategies in CRPC

Categories of resistance based on persistent hormonal signaling in castration resistant prostate cancer	Examples of biologic targets	Clinical research strategies to address persistent hormonal signaling
1. Persistent androgenic ligand synthesis or decreased ligand degradation leading to persistent AR activation	Novel androgenic synthesis enzymes: STAR, CYP17A1, AKR1C3, HSD3B2, SRD5A1/2, CYP11A1, RL-HSD, RODH4 Novel androgenic catabolic enzymes: AKR1C1/2, UGT2B15, CY3A5/A7	Higher dose abiraterone, abiraterone with food (CYP17, HSD3B2), other CYP17 inhibitors (orteronel, TOK001), statins, Vitamin D3, CYP27 activation Indomethacin or analogs: AKR1C3 Estrogenic agents, statins Anti-progestin agent: CYP11A1 inhibitors, mifepristone Targeted delivery of catabolic enzymes
2. Persistent AR activity • Persistent wild-type AR and amplified AR • AR mutations • AR deletion variants • C-terminal splice variants • Altered AR co-activator/repressor complex • Nuclear AR translocation • Altered downstream effectors of AR function • Altered upstream non-androgenic inducers or enablers of AR activity	WT AR F876L antagonists N-terminal AR or DNA binding domain ASF/SF2, U2AF65 Microtubule and motor proteins AR/AR-v effectors: proliferation (UBE2C), invasion (N-cadherin), post-translational AR modifications ERG, PARP, Rb, PI3K/Akt, beta-catenin, src, FOXA1, HER2/3, EZH2, DAB2IP	ARN-509, ODM-201 Selective AR degraders (SARDs) (i.e., galeterone, others) DR103, DR105, DR106 N-terminal AR inhibitors: EPI-001 and analogs, D2, compound 30, siRNA AR Spliceosome inhibitors Co-activator inhibitors Taxanes, dynein inhibitors, HSP90 or HSP27 inhibitors N-cadherin antibodies, plasticity inhibitors, cell cycle checkpoint inhibitors (CDK4/6), REVERB-A, HDAC-3, HOXB13, or lncRNA, AR phosphorylation, acetylation, methylation PI3K/Akt/mTOR inhibitors, dasatinib, lapatinib, PARP inhibitors, ERG inhibitors, EZH2 (non-PRC2) inhibitors, Rb/E2F inhibition, copper transport
3. Hijacking of the AR transcriptome	Glucocorticoid Receptor	Selective GR antagonists delivered specifically to tumor cells
4. Non-nuclear AR functions (cytosolic/membrane)	Src kinase	Dasatinib or src kinase inhibitors
5. AR-independent pathways	N-myc or AURKA amplification	Platinum-based cytotoxic therapy for NEPC

adenocarcinomas [55]. This has led to the development of Aurora Kinase A inhibitor therapy for patients with NEPC, currently in phase II clinical trials. NEPC frequently have loss of Rb and overexpression of the epigenetic regulator EZH2, indicating their genomic complexity and likely need for combination treatment strategies [56].

Finally, CTCs have also been used to investigate genomics of CRPCs [96]. In particular, a novel isolation technique of fluorescence activated cell sorting has been used to isolate prostate CTCs from 9 patients. Genomic analysis subsequently found gains in 8q, loss in 8p, and amplification of the AR gene [96]. These mutations suggest that CTCs have the potential to provide genomic information on a patient's tumor in a noninvasive method.

Although there is much genomic and epigenomic information regarding CRPC, it remains necessary to catalog and correlate molecular

genotypes with histologic subtypes, disease course, and outcomes after systemic therapies. These molecular aberrations are currently in the background in the clinical states of CRPC but are not currently used in the clinic to predict for treatment response and thus do not have an impact currently on treatment selection or sequencing. The challenge in the next phase of research involving men with CRPC lies in linking molecular alterations with clinical states of CRPC, potentially moving toward a molecular taxonomy prognostic for survival and predictive biomarkers for rational treatment selection.

Conclusions

The majority of men with mCRPC have a stepwise progression through clinical states of CRPC, progressing from a non-metastatic disease state to metastatic, from no systemic treatment through each line of subsequent therapy. Some men present with metastatic disease at the time of diagnosis, which is a rare event in the era of PSA screening, but a disease state that may reemerge as practitioners reduce prostate cancer screening. Throughout the clinical states of CRPC, clinical characterization of patients with respect to prior therapies, sites of metastases, histologic subtype, symptom burden, and serum biomarkers should be utilized to provide important prognostic and potential predictive clinical information to patients. These clinical states should also inform on clinical research trial designs and the new Prostate Cancer Working Group guidelines. As the disease evolves from hormone-dependent prostate cancer to ARIPC in some men, the development of biomarkers and tools to define these transitions and new states will be imperative (Table 2.2). These clinical characteristics often have prognostic impact, as determined through multiple nomograms predicting survival outcomes in CRPC, and may help guide treatment selection based on anticipated disease course and aggressiveness. Some of these clinical characteristics such as Gleason grading, PSA levels, neuroendocrine subtype, bone biomarkers, and LDH levels may also have predictive value for response to specific therapeutic agents.

CRPC has become well-characterized molecularly in recent years, but we lack sufficient data to connect molecular alterations with clinical states, and unlike in other solid tumors, the sequencing of molecular changes in CRPC is not well delineated or connected with specific treatments. The challenge of future research will be to provide a molecular taxonomy for prognostication as well as for selection of appropriate therapeutic agents.

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Molecular Mechanisms of Prostate Cancer Progression After Castration

3

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Almost all prostate cancers acquire an addiction to androgenic hormones during disease development. In the normal prostate, the testicular androgen testosterone (T) is converted to the more potent androgen dihydrotestosterone (DHT) to promote gland secretory function. Androgen regulation of prostatic differentiation is then accomplished by DHT binding to an intracellular androgen receptor (AR), which triggers a cascade of events culminating in translocation of the receptor into the cell nucleus and *trans*-activation of key differentiation genes, including *KLK3* (encoding prostate-specific antigen [PSA]) and *TMPRSS2* [1–3]. Prostate cancer cells become addicted to this signaling pathway by co-opting the AR to drive malignant behavior(s). In doing so, the cells maintain a caricature of a secretory cell phenotype, producing PSA and secreting it into the bloodstream rather than the ejaculate. At the same time, the cells are able to use AR to escape the limits of terminal differentiation. For this reason, the lowering of circulating androgen levels by treatment with bilateral orchiectomy, estrogens, or gonadotrophin releasing hormone (GnRH) analogs has long been used to treat advanced prostate cancers [4]. In nearly all cases, this maneuver results in a fall in serum PSA levels and an improvement in symptoms attributable to prostate cancer. Unfortunately for nearly all men, inexorable progression of disease to "castration-resistant prostate cancer (CRPC)" ensues. Emerging evidence suggests that CRPC comprises a heterogeneous collection of cancers, some cases with an ongoing addiction to AR signaling, potentially treatable with new drugs like abiraterone and enzalutamide, and other cases that have become AR-independent [5]. In this chapter, the molecular mechanisms responsible for these CRPC phenotypes will be reviewed.

Gene Fusions and Prostate Cancer Dependence on AR

Somatic chromosomal translocations and deletions creating gene fusions appear most likely responsible for subverting AR-dependent terminal differentiation in prostate cancer cells, permitting AR signaling to foment inappropriate cell growth and survival, invasiveness, and metastasis. The most common such genome alteration, generating a fusion between *TMPRSS2*, an AR-regulated prostate differentiation gene, and *ERG*, an ETS family transcription factor gene, has been found in up to half of prostate cancer

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F. Saad and M.A. Eisenberger (eds.), *Management of Castration Resistant Prostate Cancer*, Current Clinical Urology, DOI 10.1007/978-1-4939-1176-9_3,

cases [6–8]. The resultant dysregulated *ERG* expression directly endows prostate cancer cells with malignant properties such as invasiveness [9, 10]. In addition, ERG also indirectly undermines AR-dependent differentiation by interacting with AR at selected sites in the genome, interfering with AR *trans*-activation and allowing *trans*-repression via activation of EZH2, the polycomb repressor component endowed with H3K27 methyltransferase activity [11]. This action of ERG does not appear to reflect a general antagonism of AR signaling *per se*: in the setting of PTEN loss, which otherwise tends to result in a general dampening of AR target gene expression in prostate cells, ERG augments the general output of the AR signaling pathway [12, 13].

Forced ETS transcription factor expression in mouse prostate cells carrying disrupted *Pten* genes leads to highly penetrant invasive adenocarcinoma [13]. In this setting, the ETS factors collaborate with AR to increase the expression of many genes regulating invasion/migration, angiogenesis, and cell death [13]. Thus, fusion genes creating AR-regulated ETS factors perturb AR signaling in prostate cancer cells in a nuanced manner, preventing terminal differentiation while permitting inappropriate activation of genes associated with malignancy. In this way, prostate cancer cells are addicted to the AR, which becomes needed both for ETS fusion gene expression and for the collaborative regulation of other malignancy genes. Not surprisingly, this addiction may be difficult to shake, as the cooperation between AR and ETS factors appears to confer robust tolerance to the deleterious consequences of additional somatic gene defects, such as inappropriate activation of PI3K-signaling accompanying *PTEN* loss, that might be acquired during prostate cancer progression, even to CRPC.

ERG is not normally expressed in prostatic epithelial cells; its appearance in such cells almost always reflects a somatic gene accident. The translocations and deletions allowing the AR-stimulated expression of ERG (and other cancer genes) in prostate cancer cells bring the androgen response element (ARE)-containing DNA sequences in the promoter and enhancer regions of *TMPRSS2* (and other AR-regulated genes) into continuity with *ERG* coding sequences [6, 8]. Remarkably, such chromosomal rearrangements appear to be triggered by AR itself. To initiate transcription of target genes, ligand-bound AR builds a transcription complex by engaging co-activators and by altering chromatin conformation. As part of this process, AR binds TOP2B, a DNA topoisomerase capable of double strand passage, to prevent tangling during DNA template looping and migration to transcription "factory" sites in the cell nucleus [14]. TOP2B function is vital to the initiation of transcription at AR gene targets, as knockdown of TOP2B expression or inhibition of TOP2B enzymatic activity prevents AR-dependent gene expression [14]. When it recruits TOP2B to the transcriptional regulatory region of genes like *TMPRSS2*, AR tends to stimulate TOP2B-mediated DNA double strand breaks that can be substrates for illegitimate recombination upon repair by the non-homologous end joining (NHEJ) pathway [14, 15]. TOP2-triggered DNA strand breaks have been implicated in the generation of gene fusions involving the *MLL* gene in treatment-associated acute myeloid leukemia (t-AML; see [16]). In this setting, enzyme dysfunction was likely induced as a consequence of exposure to TOP2-targeted anti-neoplastic drugs. However, the chromosomal rearrangements in prostate cancer cells may arise as a result of a more intrinsically error prone process. In model studies using prostate cancer cells, AR-triggered TOP2B activation can promote recombination at or near ARE sequences in AR target genes and create *TMPRSS2–ERG* fusion transcripts [14].

The contributions of AR and androgen signaling to the malignant phenotype of prostate cancer cells cannot be overstated. AR acts to induce translocations and deletions engendering gene fusions [14]. The dysregulated products of fusion genes then undermine AR-associated terminal differentiation, collaborate with AR to activate tumorigenic pathways, and prevent cell death associated with oncogenic stress, and reinforce AR-transcriptional output during prostate cancer progression [9–13]. In this way, AR action both promotes genetic instability and malignant behavior, and fusion genes facilitate co-opting of

Table 3.1 Molecular mechanisms of castration-resistant prostate cancer

Phenotype	Molecular basis	Mechanism	Consequence
AR-addicted	Adrenal or intra-tumoral androgen biosynthesis	Androgens produced sufficient to activate AR	Responds to androgen biosynthesis inhibitors and selected AR antagonists
	AR mutation	Alters ligand specificity Increases ligand promiscuity Triggers antagonist-to-agonist switch	May respond to selected AR antagonists
	AR over-expression	Increases ligand sensitivity Increases ligand promiscuity	Often responds to selected AR antagonists
	AR splice variant	Provides ligand-independent receptor function	Will not respond to AR antagonists
	AR post-translational modification	Increases receptor activity	May respond to AR antagonists
Non-AR-addicted (AR pathway-independent prostate cancer or APIPC)	Neuroendocrine prostate cancer (NEPC)	Driven by *N-MYC*, *AURKA*, and other genes	Will not respond to androgen biosynthesis inhibitors or AR antagonists
	Other	Not known	Will not respond to androgen biosynthesis inhibitors or AR antagonists

AR signaling to maintain the cancer phenotype. This type of mechanism predicts that interference with AR function should be deleterious to prostate cancer cells, underscoring the well-recognized benefit of androgen deprivation to prostate cancer treatment, and that progression to CRPC could conceivably proceed via two different routes: (1) by maintaining AR signaling in some manner, or (2) by through the development of a molecular escape mechanism from AR addiction (Table 3.1).

The Molecular Biology of AR Function

AR is a ligand-dependent transcription factor encoded by a single gene with 8 exons located at Xq11-12. The receptor is a member of the nuclear receptor superfamily group that also contains the glucocorticoid, mineralocorticoid, and progesterone receptors 3 [17]. The physiologic AR protein shows marked inter-individual differences in size as a result of variable polyglutamine and polyglycine repeats. These differences may affect receptor function, with both increased transcriptional *trans*-activation and increased prostate cancer risk seen in association with AR containing shorter polyglutamine repeats [18, 19]. AR structure can be considered in terms of mapped functional domains, including a ligand binding domain (LBD) ensuring selective activation by androgenic hormones, a hinge domain, a DNA binding domain (DBD) permitting binding selective binding to ARE sequences, and an N-terminal domain; critical regions for transcription *transactivation* (activation function or AF regions) are located both in the LBD and in the N-terminal region [1]. In the absence of androgens, the receptor is sequestered in the cytoplasm via an interaction with chaperone proteins. Hormone binding triggers a cascade of events starting with a change in AR conformation which results in liberation from chaperones, dimerization, and ingress into the cell nucleus [1].

The arrival of the ligand-bound, activated, AR in the cell nucleus attracts a myriad of transcriptional co-regulators, including histone acetyltransferases, histone demethylases, SWI/SNF proteins, poly(ADP-ribose) polymerase (PARP), and as mentioned, TOP2B, along with as many as 200 or more other proteins represented in several complexes [1, 14, 20, 21]. These complexes then act to modify chromatin proteins so as to sculpt an active chromatin conformation capable of loading RNA polymerase II at target genes. The activated AR is competent to directly engage

co-regulatory proteins containing an FxxLF amino acid motif upon ligand binding as a result of the movement of helix 12 in the LBD to create a hydrophobic pocket (AF-2 [22]). The AR itself has an FQNLF amino acid sequence within its N-terminal domain, and androgen binding to the receptor can trigger a dimeric N-terminal to C-terminal conformation by virtue of interactions of FQNLF with the unveiled hydrophobic region in the LBD [20]. AR complexes bind genomic DNA not only at ARE sequences located in proximal promoters of genes but also at enhancer elements located far upstream, in introns, and in 3' untranslated regions [23]. Once in the cell nucleus, activated AR can also modulate a number of genome functions, including facilitating the activation or repression of other genes and promoting licensing of DNA replication origins [24]. Of note, ligand-bound, activated, AR remaining in the cytoplasm has been reported to interact with kinases such as SRC to initiate additional signaling programs [25]. The transcription output attributable to activated AR in normal prostate cells includes differentiation genes such as *KLK2*, *KLK3*, and *TMPRSS2*, while in CRPC cells, AR also tends to promote expression of cell-cycle genes such as *CDC20*, *UBE2C, CDK1*, and *ANAPC10* [23].

Molecular Mechanisms of Maintained AR Addiction in Many Cases of CRPC

Despite frequent initial beneficial treatment responses to androgen deprivation therapies that lower circulating testosterone levels to <50 ng/mL, CRPC tends eventually to emerge and progress to ultimately threaten life. Intriguingly, CRPC is most often heralded by progressive rises in serum PSA, a biomarker requiring activated AR function in prostate cancer cells [26]. This implies that there is ongoing AR signaling in these cancers, hinting they likely have remained addicted to AR [5]. How does this addiction persist? One mechanism involves ongoing production of androgens, either by adrenals or by the cancer itself, which persists despite ablation of testicular androgen biosynthesis [27, 28]. At cancer sites, T and DHT can be produced at levels sufficient to activate AR either by conversion of adrenal androgen precursors or by new synthesis using CYP17 [27, 28]. This process can be antagonized by ketoconazole, an antifungal drug capable of inhibiting CYP17 when administered at high doses, and by abiraterone acetate, a pregnenolone analog now approved for treatment of CRPC based on survival prolongation in randomized clinical trials [29]. A second CYP17 inhibitor, orteronel (TAK-700), is in advanced clinical development [30].

Another AR-addicted CRPC phenotype can be attributed to new *AR* mutations, not present at disease presentation, which encode receptors with altered ligand specificity. These cases tend to arise in the setting of treatment with "first-generation" anti-androgens, such as flutamide and bicalutamide, and may be responsible for an "anti-androgen withdrawal" syndrome, where men with disease progression despite the combination of androgen deprivation and anti-androgen administration show improvement upon cessation of anti-androgen treatment but maintenance of androgen deprivation [6, 31, 32]. Such *AR* mutations do not arise commonly in the setting of androgen deprivation alone. Even in the absence of *AR* mutations AR-addicted CPRC cases may arise as a consequence of ongoing AR activation stimulated by growth factor signaling pathways capable of creating post-translational modifications of AR or its co-activators [1, 5, 26].

In a study of CRPC arising among several different human prostate cancer xenografts propagated in immunodeficient mice, the most consistent molecular finding was that the *abundance* of AR was increased, leading to augmented transcriptional *trans*-activation at lower androgenic hormone levels and in response to a more promiscuous collection of ligands [33]. Amplification of *AR* has been reported in as many as 80 % of CPRC cancers, with some 30 % exhibiting marked gene amplification, which may account for some instances of AR over-expression [34, 35]. This high AR expression phenotype was exploited in the discovery of the "second-generation" anti-androgens enzalutamide and ARN-509, drugs

that appear capable of interfering with AR function despite high-level AR expression in prostate cancer cells that otherwise do not respond to "first-generation" anti-androgen treatment [36, 37]. When compared to the "first-generation" anti-androgens, these new agents exhibit less mixed agonist and antagonist activity when docking to the LBD, triggering a different location of helix 12. The drugs stop AR activation almost entirely, before receptor trafficking to the cell nucleus and binding to ARE sequences [36, 37]. Thus far, enzalutamide has gained approval for CRPC treatment based on randomized trial data showing a survival benefit and ARN-509 trials are ongoing [38].

Predictably, the growing use of enzalutamide for CRPC has fostered the emergence of cancers resistant to "second-generation" anti-androgens. In many such treatment-resistant cases, the AR signaling addiction appears maintained. Model studies of the acquisition of "second-generation" anti-androgen resistance using LNCaP prostate cancer cells have revealed a new mutant AR with an F876L amino acid change in the receptor LBD [39–41]. Enzalutamide was able to bind the F876L-AR with 48-fold greater affinity that wild-type AR; ARN-509 also bound F876L-AR [39]. When the F876L-AR was introduced into prostate cancer cells, enzalutamide and ARN-509 acted as agonists rather than as antagonists, driving AR target gene expression and stimulating prostate cancer cell growth in vitro and in vivo [39, 40]. The F876L-AR mutation has been detected in men with progressive CPRC despite "second-generation" anti-androgen treatment. When plasma DNA from a phase 1 clinical trial of ARN-509 for CPRC was assayed for *AR* mutations, 3 of 18 men with progressive serum PSA increases despite treatment were found to have a C to A missense change at *AR* nucleotide 2628 encoding the F876L-AR [39]. Already, drug discovery efforts are underway for "next-generation" anti-androgens which can inhibit signaling by F876L-AR, raising the possibility that as long as CRPC remains addicted to AR with an intact LBD, small molecule antagonist drug therapy may be feasible [41].

A final phenotype of maintained AR addiction in CRPC may be mediated by *AR* splice variants that encode receptors without LBDs that can nonetheless act to promote target gene transcription [42–46]. The variant *AR* transcripts contain deleted or cryptically inserted exons. Some such transcripts may be generated by defective *AR* genes arising in association with *AR* amplification or other *AR* rearrangements [47]. However, the majority of the variant *AR* transcripts likely arise a result of some sort of perturbation in transcription initiation and elongation rates that accompanies androgen deprivation [48]. The resultant receptors contain truncated C-terminus, with an intact N-terminal domain and DBD, but not a functioning LBD. Usually, in prostate cancer cells, the level of variant *AR* mRNA tends to be far less than that of full-length *AR* mRNA. However, this may underestimate the expression level of the truncated AR forms encoded by variant *AR* transcripts, which may be as high as 30 % or more of AR protein [49]. Forced expression of one such truncated receptor, AR-V7 which lacks an LBD and contains 16 amino acids from a cryptic exon, triggered expression cell-cycle regulatory genes in prostate cancer cells, whether or not androgens were present [50]. This *AR* variant, which has been detected in men with CRPC, can drive the growth of prostate cancer cells in the absence of androgenic hormones. Also, while both full-length AR and AR-V7 tend to promote expression of prostate differentiation genes like *KLK3* and *TMPRSS2*, there are tantalizing differences, as yet unexplained, in the patterns of genes induced by each receptor [50]. Several of the *AR* variants so far detected also appear to be expressed in CPRC cells and to possess the propensity to propagate ligand-independent signals, while others may not act in this manner [20]. Nonetheless, data are accumulating to suggest that *AR* splice variants may contribute both to abiraterone resistance and to enzalutamide resistance in CRPC [51].

CRPC Abandonment of AR Addiction

Progression to CRPC, in part driven by highly unstable genomes and epigenetic regulation and in part driven by therapeutic pressure, can result

in a phenotype/genotype that is entirely independent of AR signaling, a tumor state that has recently been operationally termed *A*ndrogen Receptor *P*athway-*I*ndependent *P*rostate *C*ancer (APIPC) [52]. One type of APIPC can be characterized by features such as loss of PSA production, lytic bone metastases, hypercalcemia, or widely disseminated visceral metastases that are otherwise uncommon complications of systemically advanced prostate cancer. This prostate cancer cell phenotype, often referred to as neuroendocrine prostate cancer (NEPC), does not manifest AR expression or AR signaling, representing an escape from AR addiction [53]. Molecular "archeology" studies of NEPC strongly suggest that most such cases evolved from AR-addicted prostate cancers, as many have been found to contain *TMPRSS2–ERG* rearrangements [54]. Despite the presence of the rearrangements, the absence of AR signaling prevents *TMPRSS2–ERG* fusion mRNA or ERG protein expression. AR-independent NEPC cases appear instead to contain other genome alterations, such as amplification of *N-MYC* and *AURKA* [55]. For this reason, the NEPC variant of CRPC does not respond to treatments targeted at the AR signaling axis. Instead, the use of cytotoxic chemotherapy, usually with platinum compounds, is often attempted [56]. Ongoing clinical trials are assessing whether AURKA inhibitors might provide benefit to men with NEPC and AURKA amplification with AURKA over-expression. Of concern, though this AR-independent NEPC variant of CRPC was once thought to be rare, the frequency with which it appears may be increasing with the introduction of androgen biosynthesis inhibitors and "second-generation" anti-androgens [57]. CRPC progression to NEPC is clearly dangerous, autopsy studies of life-threatening CRPC have hinted that as many as 25 % or more of men who die with prostate cancer show signs of NEPC [58].

Recent evidence suggests prostate cancers transitioning from an AR-driven to an APIPC state are not obligated to progress via a neuroendocrine phenotype, but may be dependent on alternative survival pathways. For example, as many as 80 % or more of CRPC cases show increased FGF8 expression, and cases with amplification of *FGFR2* have been identified [59, 60]. As men live longer with CRPC by responding to the new androgen biosynthesis inhibitors and better anti-androgens, more subtypes of APIPC are expected to emerge [61].

The Molecular Pathogenesis of Lethal Prostate Cancer: Is the Emergence of CRPC Inevitable?

To explain anti-neoplastic drug resistance, Goldie and Coldman applied principles first elaborated by Luria and Delbrück in classic studies of the resistance of bacteria to phage lysis to discriminate the contributions of spontaneous versus induced mutagenesis [62, 63]. Of course, all human cancer cells arise as a consequence of somatic genome errors, and most tend to show an increased propensity for genome instability. Cancer genome sequencing and other genome analyses have disclosed abundant base changes, insertions, deletions, amplifications, chromosome copy number changes, and DNA methylation differences in prostate cancers and other human cancers [64]. Early estimates hint that prostate cancer genomes contain on the order of 3,866 base changes, 108 rearrangements, and 5,408 differentially hypermethylated sequences [65, 66]. Ongoing genome instability can clearly result in cancer treatment resistance. Studies of cancer cell resistance to anti-metabolites and cytotoxic agents have consistently implicated a spontaneous mutation process of some sort as responsible for the emergence of resistant cancer cell clones among cells that were otherwise sensitive to the drugs. Such spontaneous mutation rates have been reported to be high as 1 in 10^4 per cell/generation in model studies [67]. For prostate cancer, both spontaneous and induced genome alterations probably contribute to disease progression to castration-resistance. The propensity for AR to recruit TOP2B to the regulatory regions of its target genes in prostate cancer cells and trigger directed chromosomal translocations may be a source of induced genome defects that can drive CRPC [14]. As an example, AR–TOP2B-associated DNA double strand breaks might conceivably promote *AR* amplification

and AR over-expression. Prostate cancers have been proposed to evolve via rare widespread chromosomal rearrangement events termed "chromoplexy," especially in cases showing AR-regulated fusion genes [68]. In contrast, spontaneous mutagenesis is likely responsible for the appearance of F876L-AR in men with CRPC treated with "second-generation" anti-androgens.

Genome-scale analyses of CRPC recovered at autopsy have consistently implicated a single lethal clone and its progeny as responsible for metastatic dissemination and ultimate life-threatening progression. However, ongoing genome instability in progeny of the lethal clone has been a consistent finding, suggesting that many, if not most, prostate cancers will be able to progress despite currently available treatment with androgen deprivation, inhibition of androgen synthesis, "second-generation" anti-androgens, and taxane chemotherapy, as well as to ultimately evade any future attempt at targeted therapy. In one study, genome copy number analysis showed greater similarity in losses and gains among metastatic cancer deposits in one case versus another, but distinct differences among the metastases in each case [69]. An analysis of somatic DNA hypermethylation changes capable of affecting gene expression delivered a similar result [70]. Intriguingly, in this study loss of cytosine methylation, evident in all metastatic prostate cancers, showed marked differences in every metastatic lesion, even within a single CRPC case, regardless of metastatic site. Hypomethylation appears to vary cell-to-cell in metastatic prostate cancer [67]. In addition to reducing the fidelity of suppression of normally silenced embryonic genes, like *NY-ESO* and others, this epigenetic instability may augment genetic instability in prostate cancer cells via activating retrotransposons and reducing chromatin barriers to repeat sequence recombination [70, 71].

Conclusions

The initial sensitivity of prostate cancers to androgen deprivation likely reflects a redirection of AR signaling from terminal differentiation toward maintenance of a malignant phenotype. This prostate cancer cell addiction to AR forms the basis for androgen deprivation therapy, the most widely used systemic treatment for advanced prostate cancer. Unfortunately, prostate cancers show enough genetic and epigenetic instability that disease progression to castration-resistance is inevitable. CPRC that has remained addicted to AR, particularly if the AR contains an intact LBD, is often amenable to treatment with androgen synthesis inhibitors and "second-generation" anti-androgens that selectively target the receptor LBD. CRPC that is driven by truncated AR encoded by splice variant *AR* transcripts, or that has escaped AR addiction, tends to be refractory to such drugs. AR-independent prostate cancers appear to be highly aggressive and difficult to treat with the current armamentarium of available anti-neoplastic drugs.

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New Imaging Modalities

4

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Introduction

Prostate cancer is the mostly commonly diagnosed cancer among men, and the second leading cause of cancer deaths in men in the U.S.A. [1]. Prostate cancer treatment requires precise information about the local and distant extension of disease in patients with high-risk prostate cancer. Salvage treatment options for focal salvage therapy in particular require robust visualization of local extension of cancer, lymph node involvement, presence and extent of distant metastasis [2, 3]. Salvage lymph node dissection is also being offered to patients with nodal recurrence of prostate cancer [4, 5]. Determination of degree of lymph node involvement has a pivotal role in planning new therapeutic options in advanced disease. Treatment of castration-resistant prostate cancer (CRPC) depends on several factors such as the presence of distant metastases [6]. Hematogenous spread of prostate cancer frequently involves bone (90 %), lung (46 %), liver (25 %), pleura (21 %), and adrenals (13 %) [7]. Currently, skeletal metastases are usually evaluated by bone scintigraphy (BS) (planar and single photon emission computed tomography [SPECT]) and positron emission tomography (PET), while soft-tissue metastases are mainly detected by Computed Tomography (CT), Magnetic Resonance Imaging (MRI), and PET. Equivocal findings on BS are being investigated by dedicated CT, MRI, and standard directed radiographs or targeted X-rays (TXR) [8].

In the future, the paradigm of cancer care will involve risk adapted patient-specific therapy designed to maximize cancer control while minimizing the risk of complications and side effects. Currently available and emerging non-invasive molecular imaging techniques promise to play an important role in prostate cancer care in the future. Novel techniques are being developed using biomarkers to non-invasively probe cellular and molecular signaling pathways involved in prostate cancer and disease progression [9].

With the development of a widening choice of therapeutic options, the need for improved imaging for diagnosis and therapy response of prostate cancer is becoming increasingly relevant. In the recent past the development of docetaxel-based chemotherapy improved survival in patients with CRPC compared with mitoxantrone [10]. Since 2010, the therapeutic options for the treatment of metastatic and advanced prostate cancer have now greatly improved for patients with metastatic CRPC. There are now a myriad of novel anticancer drugs for CRPC including the novel taxane cabazitaxel, the immunotherapy sipuleucel-T,

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F. Saad and M.A. Eisenberger (eds.), *Management of Castration Resistant Prostate Cancer*, Current Clinical Urology, DOI 10.1007/978-1-4939-1176-9_4,

the anti-androgen CYP17 inhibitor abiraterone, the novel androgen-receptor antagonist enzalutamide and the alpha-emitting radioisotope radium-223. In addition to these newly approved therapies, there are a large number of targeted therapies that are being assessed at different phases of clinical trial development [10, 11].

An important issue that arises when evaluating these new therapeutic agents, as well as combinatorial studies of these agents, is the need for robust biomarkers for optimization of therapy development and clinical management of this growing therapeutic armamentarium. Current strategies to evaluate disease response and progression employ a combination of parameters, including rising serum prostate specific antigen (PSA) levels, CT and BS imaging criteria, and worsening clinical symptoms which are limited in their sensitivity for monitoring disease progression. Conventional imaging modalities for prostate cancer such as computed tomography (CT) and technetium-99m (^{99m}Tc)-based BS have been limited by low accuracy, low specificity, and inability to detect nodal disease for BS. Newer imaging methodologies utilizing SPECT and PET-based radiopharmaceuticals for prostate cancer detection promise improved detection of bone and lymph node metastases (LNM) compared to current conventional imaging modalities. Despite advances in therapy options, the diagnostic landscape has remained relatively unchanged however. Newer imaging modalities may render a substantial proportion of patients with CRPC thought to be non-metastatic to be in fact metastatic based on the improved sensitivity of these imaging modalities. This raises the question whether earlier detection of metastatic disease in prostate cancer will ultimately result in any clinical benefit. An emerging variety of imaging techniques raise the need to reassess the optimal methodology and timing of metastasis detection of various imaging techniques [12, 13].

In this chapter, we review the role of various imaging modalities in detection of local recurrence, lymph node involvement, and distant metastasis of prostate cancer, with particular focus on PET and MRI. Furthermore, the role of imaging in the assessment of therapeutic response in patients with CRPC is also discussed.

Positron Emission Tomography

Role of PET in Prostate Cancer

Functional imaging modalities such as PET are uniquely able to provide a non-invasive three-dimensional spatial imaging and functional read out of a particular relevant tumor metabolic or molecular signal with the ability to assess tumor heterogeneity within the whole patient. The main clinical indications for imaging can be broadly classified as: (1) diagnosis and initial staging, (2) detection of biochemical recurrent metastatic disease, (3) detection of advanced castrate resistant metastatic disease [13, 14]. Currently available PET radiopharmaceuticals for prostate cancer imaging include glucose and lipid metabolism agents as well as sodium fluoride bone detection-based imaging methodologies. Emerging radiopharmaceuticals include amino acid, androgen receptor, and prostate specific membrane antigen (PSMA)-based radiotracers [13].

Glucose Metabolism

Detection of prostate cancer using ^{18}F-FDG (FDG) by utilizing glucose tumor metabolism is limited in the prostate cancer setting for detection of primary disease and local recurrence due to its inherently low sensitivity and overlap with prostatitis and benign prostatic hypertrophy [15, 16]. FDG PET is not routinely recommended for the management of prostate cancer metastatic disease, especially for soft-tissue metastases [17]. However, when applied to the proper clinical and patient selection scenario, it has been able to demonstrate utility for detection and as a prognostic marker in patients with prostate cancer. The use of FDG PET for detection of metastatic disease in patients with PSA relapse after radical prostatectomy was demonstrated in 31 % of patients when using a threshold serum PSA of ≥2.4 and PSA velocity of 1.3 ng/mL/year resulting in a sensitivity (80 %; 71 %) and specificity (73 %; 77 %), respectively, for the PSA parameters [18]. In the treatment response setting, greater than a 33 % increase in the average

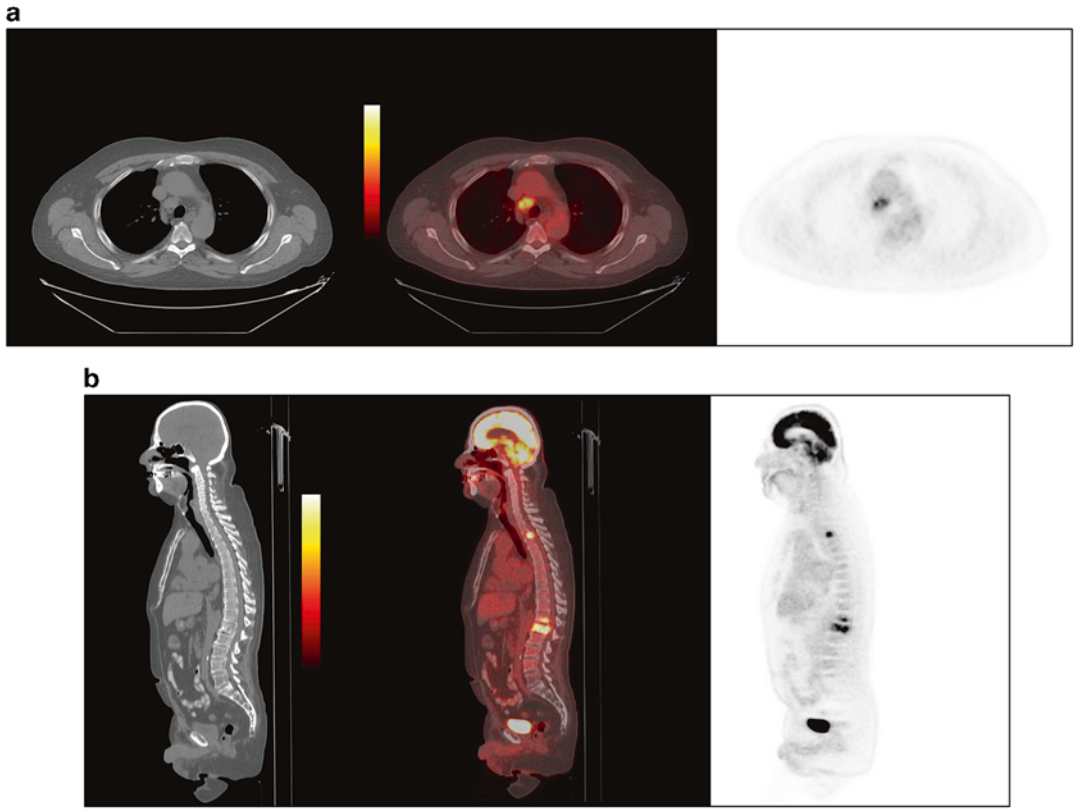

Fig. 4.1 A 66-year-old male with advanced metastatic CRPC on FDG PET/CT demonstrates an FDG-avid (**a**) right paratracheal metastatic lymph node and (**b**) vertebral body bone metastases (T3, T12, L1)

maximal FDG PET standardized-uptake value (SUV) of indicator metastatic lesions in patients undergoing therapy was able to discriminate between progressive or non-progressive disease on chemotherapy [19]. FDG PET maximal SUV ($SUV_{max)}$ in patients with progressive prostate cancer was also found to be an independent predictor of survival in multivariate analysis [20]. Figure 4.1 demonstrates an example of FDG uptake in advanced metastatic prostate cancer.

Sodium Fluoride: Bone Remodeling

The main sites of prostatic skeletal metastases are ribs, thoracic and lumbar vertebrae, and ilium of the pelvis. Other less common sites of involvement are ischium, sacrococcyx, femur, skull, cervical vertebrae, sternum, scapula, and upper limb bones [21]. The bone involvement is commonly detected by BS and standard radiographs. Other imaging modalities such as CT, MRI, PET, and PET/CT can also be used in specific circumstances based on imaging timing, costs, radiation dose, and availability [22]. The current sequential approach is BS, BS plus TXR, and addition of CT or MRI in equivocal or discrepant cases [23].

^{18}F-Sodium Fluoride (NaF) is gaining renewed interest and wider application for detection of osseous metastatic disease because of the widespread availability and improved quality of PET/CT scanners, but also because of the intrinsically higher spatial resolution of PET (compared to planar and SPECT imaging) and potential for PET quantitation of metastatic tumor burden [24]. NaF is a marker of bone perfusion and bone turnover, in which ^{18}F-Fluoride ions exchange with hydroxyl groups in the hydroxyapatite crystal of bone to form fluorapatite with higher uptake in new bone (osteoid) because of higher availability of binding sites [24]. NaF PET/CT has been reported to be a highly sensitive and specific

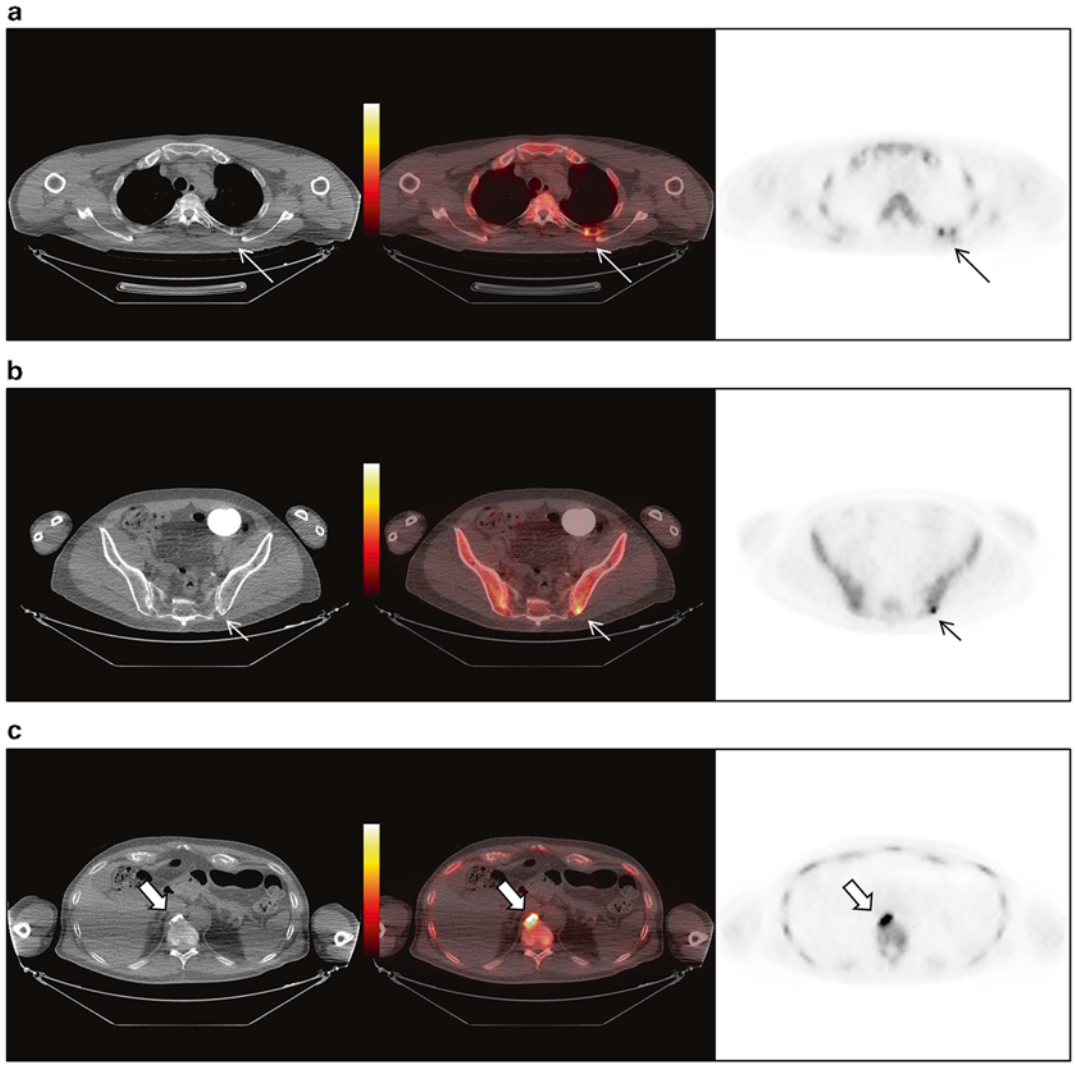

Fig. 4.2 A 68-year-old male with bone metastases on NaF PET/CT involving the (**a**) left fourth rib (*long arrow*), (**b**) left posterior iliac bone (*short arrow*), and (**c**) additional site of benign uptake at a degenerative vertebral body endplate osteophyte (*arrow head*)

modality for detection of bone metastases in prostate cancer compared to standard ^{99m}Tc-MDP (MDP) planar bone scans and MDP SPECT imaging [25]. The anatomic CT portion of the PET/CT was able to differentiate between benign and malignant lesions improving the specificity of NaF PET/CT vs NaF PET alone. Recent clinical practice guidelines for NaF PET/CT bone scans have been published by the Society of Nuclear Medicine and Molecular Imaging in 2010 [26]. A systemic review of the literature provides evidence for superior detection of bone metastases by both NaF PET and a functional tumor-based choline PET imaging agent (^{18}F-fluorocholine or ^{11}C-choline), with or without CT, compared with conventional planar MDP bone scan [27]. This review reported a sensitivity and specificity for NaF PET or PET/CT of 88.6 and 90.7 %, respectively, on a per lesion basis, and 86.9 and 79.9 % on a per patient basis. It is noted that NaF uptake post-treatment flare phenomenon, similar to that observed with MDP bone scans, has also been observed, which should be taken into consideration [28]. Figure 4.2 shows examples of NaF

PET/CT bone metastases and benign uptake in a degenerative vertebral osteophyte.

One of the benefits of NaF PET compared to ^{99m}Tc-MDP (MDP) bone scans is the potential for quantitation of bone metastatic tumor burden and treatment response. A bone scan index using standard planar MDP bone scan had been proposed as an imaging biomarker to improve the reproducibility of treatment response assessment [29]. This index aimed to quantify tumor burden as a percentage of the total skeletal mass of a reference man but there are inherent limitations based on planar imaging. PET imaging with NaF for quantitative analysis for therapy response assessment would have potentially improved benefits over more limited planar and visual qualitative MDP bone scan-based assessment methods. One report in the literature using NaF PET for monitoring treatment response of bone metastases to radiotherapy ^{223}Ra-Chloride was found to be more accurate than the visual qualitative assessment of scans, correlation with the serum-based PSA response or alkaline phosphatase activity [30]. A study of the kinetics and reproducibility of NaF PET/CT for detection and quantitation of bone metastases concluded an uptake period of 60 ± 30 min was sufficient for quantitation, with limited temporal dependence and demonstrated high tumor-to-normal tumor ratio [31]. Repeated baseline scans in this study also showed high intraclass correlations (>0.9) and relatively low critical percentage change (the value above which a change can be considered real) for these parameters, demonstrating relatively high reproducibility.

Lipid Metabolism

Choline

Radiolabeled choline PET imaging radiotracers, ^{11}C-Choline and ^{18}F-Fluorocholine, are taken up in prostate cancer cells through choline transporters and phosphorylated intracellularly by choline kinase, associated with phospholipid metabolism [32–35]. ^{11}C-Choline demonstrates rapid prostate cancer uptake, rapid blood pool clearance (within minutes), relatively small excretion in urine and relatively high diffuse liver uptake [36]. ^{18}F-Fluorocholine demonstrates higher urinary excreted radiotracer compared to ^{11}C-Choline but has the advantage of longer half-life allowing for wider clinical application through established regional radiosynthesis distribution networks without a need for an on-site cyclotron and radiochemistry facility at non-academic sites [37]. Both ^{11}C-Choline and ^{18}F-Fluorocholine have been used widely for a variety of prostate cancer imaging applications, mostly in Europe and Japan, in a large number of publications. Recently, ^{11}C-Choline was approved by the US Food and Drug Administration (FDA) in 2012 to the Mayo Clinic in Rochester, Minnesota, for production and PET imaging of patients with suspected recurrent prostate cancer after initial therapy based on elevated serum prostate specific membrane antigen (PSA) levels and noninformative BS, CT, or MRI imaging to help localize potential sites of tumor for subsequent histologic confirmation [38].

There have been several recent systematic reviews which help to compile and summarize potential clinical applications. Overall, ^{11}C-Choline PET/CT has been reported to affect therapy management in 24 % (11/45) of patients with advanced prostate cancer [39], and generally ^{11}C-Choline and ^{18}F-Fluorocholine have similar performance for detection of prostate cancer in various settings with more detailed analyses below and also reviewed [13].

The diagnostic performance of ^{18}F-Fluorocholine or ^{11}C-Choline PET or PET/CT for staging at initial diagnosis for prostate cancer has been compiled in a systematic literature review and meta-analysis by Evangelista et al. in 2013. They reported low sensitivity but high overall specificity for detection of LNM prior to prostatectomy. In their analysis of 10 selected studies, a total of 441 patients from 2000 to January with pooled sensitivity of 49.2 % (95 % confidence interval [CI], 39.9–58.4) and pooled specificity of 95 % (95 % CI, 92–97.1) was reported [40]. A major issue for the low sensitivity of these choline-based radiotracer is reportedly due to the inherent limitation of PET imaging to detect small metastatic lymph nodes less than 0.4 cm in diameter.

The utility of ^{18}F-Fluorocholine or ^{11}C-Choline PET or PET/CT for detection of prostate cancer recurrence after definitive radical prostatectomy or external-beam radiation therapy was also compiled in another systematic literature review and meta-analysis by Evangelista et al. in 2013 [41]. This study found a high sensitivity and specificity for detection of locoregional and distant metastases by choline PET or PET/CT imaging. Through their selection criteria, 19 studies were chosen with a combined total of 1,555 patients from 2000 to 2012 with a pooled data demonstrating for all sites of disease (prostatic fossa, lymph nodes, and bone) a sensitivity of 85.6 % (95 % CI: 82.9–88.1 %) and specificity of 92.6 % (95 % CI: 90.1–94.6 %), for prostatic fossa recurrence a sensitivity of 75.4 % (95 % CI: 66.9–82.6 %) and specificity of 82 % (95 % CI: 68.6–91.4 %), and for LNM a sensitivity of 100 % (95 % CI: 90.5–100 %) and specificity of 81.8 % (95 % CI: 48.2–97.7 %) [41]. They conclude that choline-based PET or PET/CT imaging can be used for the identification of lymph node recurrence, but raise concerns that due to the loss in specificity may result in unnecessary surgical treatments. The authors recommend identification of relapse in prostate cancer patients based on PSA stratification, with the strongest predictor of PET positivity based on threshold PSA-based parameters (PSA > 1 ng/mL, PSA velocity (vel) > 1 ng/mL/year, and a PSAdt < 3 months). Ongoing hormonal therapy did not limit the diagnostic accuracy for detection of metastatic disease. However, they do not recommend choline PET/CT for detection of local recurrence [41].

Another systematic review and meta-analysis of ^{18}F-Fluorocholine or ^{11}C-Choline PET or PET/CT for staging and restaging of prostate cancer was performed by Umbehr et al. [42]. They reviewed the literature up to July 2012 and selected 44 studies. The authors could not recommend Choline PET or PET/CT imaging without reservation for routine clinical use for prostate cancer imaging based on current evidence, although the diagnostic evidence was found to be higher in restaging than in staging settings. They also recommend careful selection of eligible patients based on PSA criteria to avoid false negative imaging results up front in staging as well as restaging clinical scenarios. In the staging scenario, mainly high-risk Gleason scores (8–10) and high PSA levels (≥20 ng/mL) were thought to be predictive of improved choline PET and PET/CT imaging performance. In restaging settings, minimal recurrent PSA levels (>1 ng/mL), short PSAdt (<3 mo to a maximum of 6 months), and initial tumor stage (>pT3b or pN1) were found to improve imaging detection [42].

Acetate

Acetate is another lipid-based agent incorporated into prostate cancer cells with over-expression of fatty acid synthase, a key enzyme in fatty acid synthesis from acetyl CoA [43]. ^{11}C-Acetate as a PET radiotracer was first demonstrated to detect primary prostate cancer and metastatic disease at initial staging in patients with rising PSA after radical prostatectomy [44] or after external-beam radiation therapy [45].

A systematic review of the literature with meta-analysis of ^{11}C-Acetate PET imaging in prostate cancer was performed by Beheshti et al. [46]. They reviewed all published studies up to March 2013 and selected 24 studies for meta-analysis. For primary tumor detection, pooled sensitivity 75.1 % (95 % CI: 69.8–79.8) and specificity was 75.8 % (95 % CI: 72.4–78.9). For detection of recurrence tumor, pooled sensitivity was 64 % (95 % CI: 59–69) and specificity was 93 % (95 % CI: 83–98), with higher sensitivity in patients with PSA > 1 ng/mL and in post-prostatectomy compared to external-beam radiation therapy patients [46]. Of note, studies comparing ^{11}C-acetate and Choline-based PET imaging were reported to be comparable in their analyses with low sensitivity and relatively high specificity for detection of tumor recurrence and limited value for detection of primary tumor.

When ^{11}C-Acetate was compared with MRI and prostatectomy histopathological correlation, ^{11}C-acetate PET/CT demonstrated higher uptake in tumor foci than in normal prostate tissue but PET uptake was not able to distinguish tumor from benign prostate hyperplasia nodules. In a sector-based comparison with histopathology, all tumors greater than 0.5 cm by histopathology

revealed sensitivity of 61.6 % and specificity of 80.0 % for ^{11}C-acetate PET/CT, vs sensitivity of 82.3 % and specificity of 95.1 % for multiparametric MRI (T1, T2, DWI, MRS), with the accuracy of ^{11}C-acetate comparable to that of MRI only when tumors were greater than 0.9 cm were assessed [47].

A recent large trial evaluated ^{11}C-Acetate PET/CT prior to prostatectomy for nodal staging compared with pathologic nodal status and clinical follow-up for treatment failure in 107 men with intermediate- or high-risk localized prostate cancer [48]. ^{11}C-Acetate was positive for local pelvic nodal or distant metastatic disease in 33.6 % of patients, with lymph node metastasis present histopathologically in 23.4 % of these PET-positive metastatic patients. The overall performance of ^{11}C-Acetate for detection of lymph node metastasis prior to prostatectomy revealed a sensitivity of 68.0 %, specificity 78.1 %, positive-predictive value of 48.6 %, and negative-predictive value of 88.9 %. Interestingly, ^{11}C-acetate PET detection of any metastasis in this clinical study also independently predicted treatment-failure–free survival in a multivariate analysis [48].

Androgen Receptor Imaging: FDHT

16β-^{18}F-fluoro-5α-dihydrotestosterone (^{18}F-FDHT) is an analog of dihydrotestosterone, the primary ligand for the androgen receptor (AR) which allows for non-invasive PET imaging of AR expression [49]. The first study of seven patients with progressive metastatic CRPC demonstrated ^{18}F-FDHT detection of 78 % of the lesions compared to conventional imaging with average lesion SUV_{max} value of 5.28 [50]. Another study also demonstrated ^{18}F-FDHT uptake at sites of metastatic disease, confirming AR-mediated ^{18}F-FDHT PET signal as seen with interval decreased ^{18}F-FDHT uptake from baseline (SUV_{max} decreased 9–70 %) after competition with flutamide [51].

The potential role of AR imaging as a pharmacodynamic marker in prostate cancer has been demonstrated in several recent studies. ^{18}F-FDHT and ^{18}F-FDG PET/CT imaging of a subset of 22 patients enrolled on a phase 1–2 study of MDV3100 (Enzalutamide) was able to show a reduction in ^{18}F-FDHT tumor uptake from baseline (percent SUVmax change range from 20 to 100 %) after starting treatment compatible with competition or blocking of ^{18}F-FDHT from the AR-binding site by the therapeutic agent [52]. However, these patients show a poor treatment response as only 45 % of these patients by $^{18F\text{-}FDG}$ PET/CT scans had 25 % or greater decrease in the FDG PET SUV_{max} after 12 weeks of therapy. These results position ^{18}F-FDHT as a pharmacodynamic marker of AR binding rather than a therapy response biomarker. A more recent study also utilized ^{18}F-FDHT-PET/CT imaging to measure pharmacodynamic response in a phase I clinical trial of a novel anti-androgen ARN-509 to incorporate imaging to visualize and quantitatively assess by SUV analysis the heterogeneity of AR-binding sites of metastatic disease and determine maximal AR inhibition by the study drug [53]. A clinically applicable method using ^{18}F-FDHT PET quantitative marker as a surrogate of pharmacokinetic parameter to non-invasively assess free AR concentration has been proposed for validation studies in clinical trials [54].

Amino Acid-Based Imaging: FACBC

An emerging and promising PET radiotracer for prostate cancer is anti-1-amino-3-^{18}F-fluorocyclobutane-1-carboxylic acid (anti-^{18}F-FACBC), a synthetic L-leucine amino acid analog. This agent is taken up in prostate cancer cells by amino acid transporters system (ASC transporter 2 or ASCT2) and to a lesser sodium-coupled neutral amino acid transporters but not incorporated into proteins intracellularly [55]. The initial first-in-human clinical study of anti-^{18}F-FACBC PET/CT demonstrated positive uptake in primary prostate and metastatic disease [56]. Figure 4.3 demonstrates an example of anti-^{18}F-FACBC PET/CT bone and nodal metastasis. In the subsequent clinical study, comparison of anti-^{18}F-FACBC with ^{111}In-capromab pendetide (ProstaScint™) for detection of recurrent disease in 50 men after prostatectomy or external-beam radiation therapy

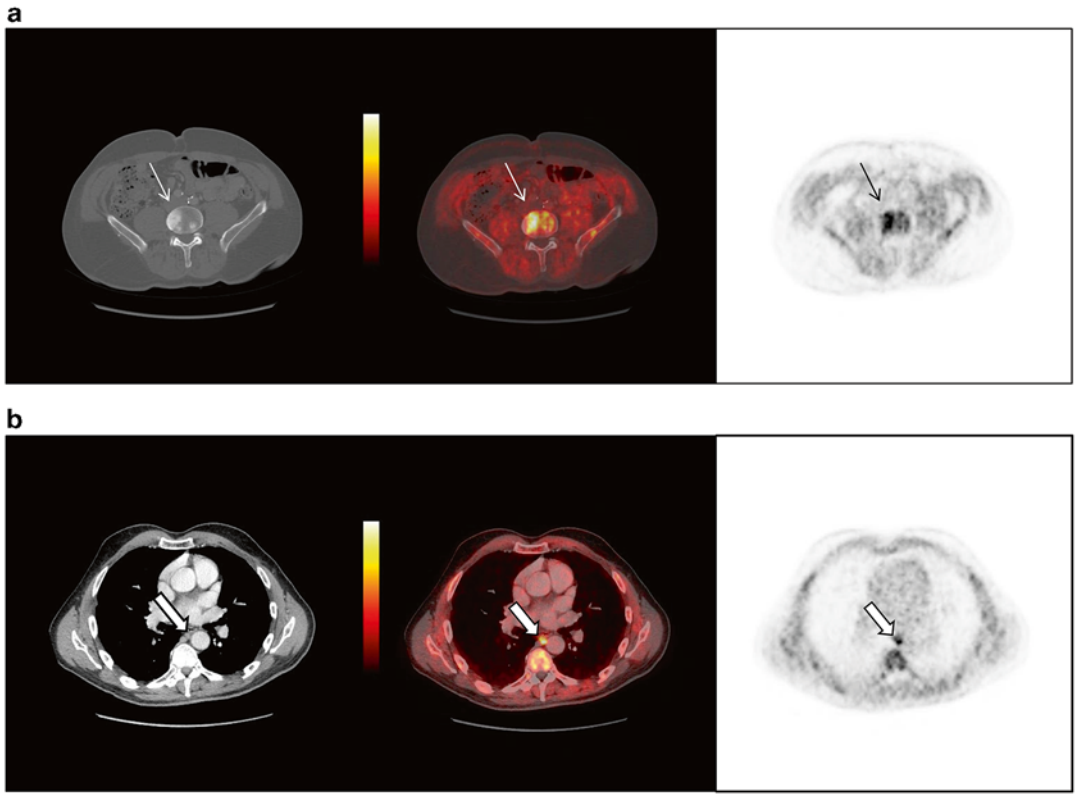

Fig. 4.3 A 64-year-old male with metastatic CRPC imaged with anti-^{18}F-FACBC PET/CT demonstrates (**a**) a L4 vertebral body bone metastasis and (**b**) small paraesophageal nodal metastasis

for prostate carcinoma revealed anti-^{18}F-FACBC PET/CT was more sensitive than ^{111}In-capromab pendetide SPECT/CT for detection of recurrent disease, especially for detection of extraprostatic recurrence. For disease detection in the prostate bed, anti-^{18}F-FACBC had a sensitivity of 89 % (95 % CI: 74–97 %), specificity of 67 % (95 % CI: 35–90 %), and accuracy of 83 % (95 % CI: 70–93 %). For the detection of extraprostatic recurrence, anti-^{18}F-FACBC had a sensitivity of 100 % (95 % CI: 69–100 %), specificity of 100 % (95 % CI: 59–100 %), and accuracy of 100 % (95 % CI: 80–100 %). A recent small study compared anti-^{18}F-FACBC with 11C-Choline PET/CT imaging for detection of recurrent disease in 15 men after definitive therapy with prostatectomy or external-beam radiation therapy. Anti-^{1}^{8}F-FACBC was found to have a higher detection rate compared to ^{11}C-Choline on a per patient (detection rate of 40 vs 20 %, respectively) and per lesion basis (6 vs 11 lesions, respectively), with all ^{11}C-Choline positive lesions also identified by anti-^{18}F-FACBC [57]. These promising initial studies will need further validation of anti-^{1}^{8}F-FACBC in larger multi-center clinical trials.

Prostate Specific Membrane Antigen-Based Imaging: PSMA

Prostate specific membrane antigen (PSMA) is a type II integral membrane protein expressed on the surface of prostate cancer cells, also known as glutamate carboxypeptidase II (GCPII) and folate hydrolase [58]. PSMA is associated with prostate cancer aggressiveness with histological studies associating high PSMA expression with metastatic spread [59–61], androgen-independence [62], and high PSMA expression levels predictive of prostate cancer progression [63, 64].

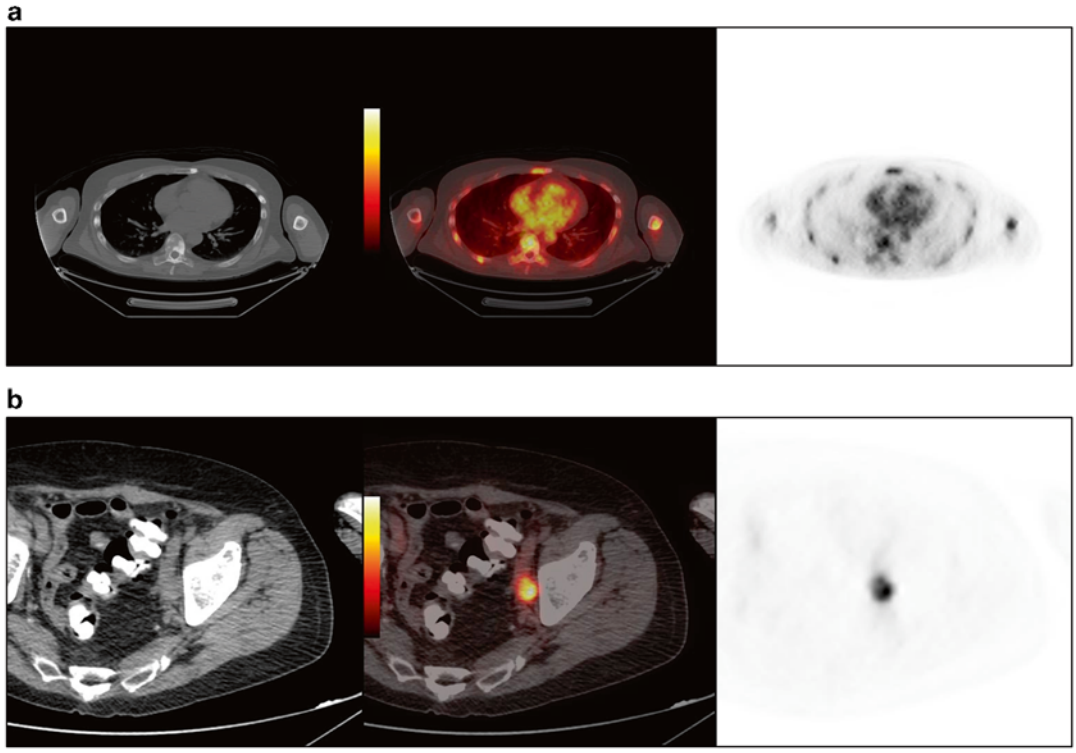

Fig. 4.4 PSMA-based ^{18}F-DCFBC PET/CT demonstrates (**a**) widespread bone metastases and "superscan" bone scan in a 62-year-old with metastatic CRPC, and (**b**) activity in a left pelvic side-wall lymph node metastasis in a 74-year-old male with CRPC

^{111}In-capromab pendetide (ProstaScint™) was first developed for PSMA-based prostate cancer imaging but demonstrated limited performance for tumor detection which may be explained by lower tumor penetration of a large sized antibody agent and binding to the less accessible intracellular epitope of PSMA [65]. An improved humanized intact antibody that binds to the more accessible extracellular epitope of PSMA, J591, has been used labeled Indium-111 and Lutetium-177 for gamma planar and SPECT/CT imaging of metastatic prostate cancer as part of a number of radioimmunotherapy trials [66–70]. A recent retrospective review of these trials presented as a research abstract ranging over a decade demonstrated J591 planar imaging reported a detection rate of 86.4 % of known lesions [67]. Next generation imaging trials are incorporating PET/CT imaging of prostate cancer with ^{89}Zr-DFO-J591 which demonstrated high prostate tumor uptake in preclinical models [71].

Smaller low molecular weight imaging agents for PSMA would have inherent advantages over intact antibodies such as rapid tumor uptake and clearance from non-target sites [58]. N-[N-[(S)-1,3-Dicarboxypropyl]carbamoyl]-4-1^{8}F-fluorobenzyl-l-cysteine (^{18}F-DCFBC) is a novel and clinically practical low molecular weight inhibitor of PSMA which in preclinical studies demonstrated high specific localization to PSMA-expressing prostate cancer xenografts [72]. An initial first-in-man study of ^{18}F-DCFBC PET/CT was able to demonstrate uptake at sites of bone and lymph node metastatic disease detected at two hours after injection, with ^{18}F-DCFBC PET detecting more lesions than corresponding conventional imaging modalities (CT, bone scan) [73]. These promising findings are undergoing further validation in ongoing clinical trials

evaluating ^{18}F-DCFBC PET/CT in primary and metastatic prostate cancer. Figure 4.4 demonstrates examples of positive detection of metastases by ^{18}F-DCFBC PET/CT in men with CRPC seen with widespread bone and another with a large left pelvis nodal metastasis. Another fluorine-18 labeled low molecular weight PSMA targeted PET radiopharmaceutical, BAY 1075553, has been published recently with preclinical validation [74].

Other radiolabeled PSMA-based radiotracers for PET imaging including a Gallium-68 labeled PSMA-based low molecular weight radiopharmaceuticals have also been radiosynthesized [75]. Afshar-Oromieh et al. reported their initial experience with PET/CT using Glu-NH-CO-NH-Lys-(Ahx)-[^{68}Ga(HBED-CC)] (^{68}Ga-PSMA) which also was able to detect prostate carcinoma relapses and metastases at high signal-to-background at 1 h after radiopharmaceutical administration [76]. Another recent study comparing ^{68}Ga-PSMA with ^{18}F-Fluorocholine with biochemical relapse of prostate cancer in the same patients within a 10-day time window in 37 patients was able to detect more lesions and higher signal-to-background by ^{68}Ga-PSMA compared to ^{18}F-Fluorocholine (78 vs 56, respectively; $P = 0.04$) [77].

PSMA-based radiotracers have also been developed for SPECT imaging, which have lower resolution compared to PET scans but are more widely available than PET. First-in-man study of two Iodine-123 labeled small molecule SPECT agents for PSMA, ^{123}I-MIP-1072, and ^{123}I-MIP-1095 also demonstrated uptake and detection of prostate cancer in soft tissue, bone, and the prostate gland as early as 1–4 h after injection [78]. Other SPECT agents for PSMA have been radiosynthesized with technetium-99 m demonstrating high specific PSMA targeted tumor uptake in preclinical models [79–81].

An interest and potentially powerful application of PSMA imaging is as a downstream marker of androgen-receptor signaling to potentially assess for real-time assessment of tumor response to androgen deprivation therapy. Evans et al. reported in preclinical studies that PSMA can be repressed by androgen treatment in multiple animal models of AR-positive prostate cancer in an AR-dependent manner, whereas androgens can up-regulate PSMA expression [82]. In another study, assessment of androgen-receptor signaling in circulating tumor cells was able to utilize PSMA and PSA expression as markers of hormonally responsive prostate cancer to androgen deprivation therapy [83]. With the emergence of PSMA-based radiopharmaceuticals the possibility of utilizing non-invasive PSMA imaging by PET/CT or SPECT/CT as a downstream biomarker of androgen-receptor signaling may be an exciting possibility but requires careful clinical validation.

Magnetic Resonance Imaging

MRI Techniques

Morphologic MR Images

Modern MRI of the prostate applies the principles of multiparametric MRI (mMRI) utilizing morphological imaging (T1-weighted and T2-weighted) and functional imaging (diffusion, perfusion, and spectroscopy). Morphologic MR images provide anatomical detail of the prostate and local tumor staging through T2-weighted images (T2WI), and some information about presence of post-biopsy hemorrhage and lymph node involvement through T1-weighted images (T1WI). In the untreated gland, peripheral zone (PZ) tumor can be detected as low signal intensity (SI) (dark) lesion on T2WI. However, there are some conditions like prostatitis, biopsy scars or fibrosis, and treatment-induced changes that may mimic the PZ tumor or may mask the surrounding or intermixed tumor.

Detection of transition zone (TZ) tumor is challenging in T2WI because of the signal heterogeneity secondary to benign prostatic hyperplasia (BPH). The TZ tumors are typically recognized as homogeneously low SI lesion on T2WI in the absence of the low SI rim surrounding the BPH nodules. Moreover, stromal type BPH and high-grade prostatic intraepithelial neoplasia (HGPIN) may also appear similar to TZ tumors on T2WI. Also, detection of anterior TZ tumors is difficult using T2WI alone due to close proximity to anterior fibromuscular stroma [84].

Diffusion-Weighted Imaging

The study of water diffusivity in the tissue is crucial to identify the tumor lesions based on different water content and cellular density. Cancer cells induce high-density cellular microenvironment and disorganized extracellular stroma appearing as high SI on high *b*-value DWI. The Apparent Diffusion Coefficient (ADC) value as a measure of water diffusion on DWI is decreased in tumor regions due to water molecule motion restriction. DWI can also be helpful to differentiate prostatitis from tumor tissue. In addition, ADC value is negatively correlated to the tumor grade, the more cellular the tumor, the higher the restriction [85, 86].

Dynamic Contrast-Enhanced MRI

Dynamic contrast-enhanced (DCE)-MRI examines the perfusion and distribution of intravenously administered contrast agent. Tumors exhibit neovascularization secondary to high level of vascular endothelial growth factor (VEGF) expression. The quantitative parameters of DCE-MRI reflect features of tumor vascularity such as disorganized structure, arteriovenous shunting, and areas of hemorrhage [87].

Tumor tissues with increasing cellularity have smaller volume of interstitial space accounting for different pharmacokinetics for distribution of contrast agent; tumor tissues have higher and earlier enhancement, and quick washout of the contrast compared with the healthy prostate gland [88]. Quantitative study of DCE-MRI with pharmacokinetic (PK) modeling can quantify the tumor blood flow, tumor microvasculature, and capillary permeability [89].

MR Spectroscopic Imaging

Metabolic profile of tumor differs from the healthy tissue on spectroscopic imaging. The main metabolites appearing on MR spectroscopic imaging (MRSI) of the prostate are choline, creatine, and citrate. The citrate level represents the glandular component of the respective voxel. Citrate level is higher in the normal PZ and glandular type of BPH than in the stromal type of BPH. The citrate level is decreased in prostate cancer, however prostatitis or hemorrhage may also lead to decreased citrate levels on MRSI [90]. Metastatic poorly differentiated prostate cancer may show very low citrate levels [91, 92].

Prostate cancer cells have higher cell membrane surface area per cellular volume. High cellular tumor proliferation is associated with high turnover of cell membrane. Therefore, choline level, a by-product of phospholipid component of cell membrane, is increased on prostate cancer spectroscopy. In addition to each individual metabolite value in MRSI, choline-to-citrate and (choline + creatine)/citrate ratios are also representative of increased cell membrane turnover and malignant tissue and have been used as biomarkers for differentiating cancer from benign tissue.

Detection of Nodal Involvement

Currently, extended pelvic lymph node dissection (ePLND) is the gold standard for accurate detection of microscopic invasion of lymph nodes [93]. Imaging criteria for detection of LNM using CT and MRI include macroscopic enlargement of lymph nodes (size >10 mm for the short axis of an oval node or above 8 mm for the diameter of a round node). A meta-analysis comparing the performance of CT with conventional MRI showed equally poor performance with sensitivity of about 40 % and specificity of about 80 % [94]. There is no suitable size threshold to accurately detect LNM. However, the level of LNM differentiates the categorization as N or M stage, where the LNM above the level of common iliac vessels bifurcation is defined as M stage [95].

As a functional parameter, DWI can potentially be used to distinguish malignant from benign lymph node enlargement. Malignant lymph nodes typically exhibit restricted diffusion, owing to higher cellularity, enlarged hyperchromatic nuclei, and abundant macromolecular protein. In a study by Eiber et al. [96] the performance of DWI in conjunction with T2WI was investigated to detect LNM in patients with prostate cancer. Some patients had histological evidence of LNM through extended PLND and others were evaluated on clinical follow-up. It was shown that

ADC values were significantly lower in malignant nodes compared with benign nodes in both subgroups. Authors identified that an ADC cut-off value of 1.30×10^{-3} could distinguish malignant from benign nodes better than short axis size cut-off of 9 mm; sensitivity 86 vs 82 %, and specificity 85.3 vs 54.4 %, respectively. However, DWI is subject to some limitations such as frequent artifacts and limited spatial resolution. Moreover, heterogeneous cohorts and lack of ePLND as gold standard in some patients in the reported studies make the interpretation of performance of DWI challenging.

Further advances have been made by introduction of lymphotropic MR contrast agent and several studies reported results from MR lymphography (MRL). Ultrasmall superparamagnetic iron oxide (USPIO) particles are virus-sized particles which are being accumulated in mononuclear phagocytic system. Therefore, they diffusely accumulate within normal lymph nodes. The normal uptake of USPIO by the macrophages exhibits a homogenously low SI in the normal lymph nodes. In LNM, due to tumor deposits in the lymph nodes instead of expected SI loss there is focal or diffuse increase in signal signifying replacement of the normal tissue by cancer. Early studies demonstrated that MRL was much more sensitive and specific than conventional MRI at 1.5 T to detect LNM (90.5 vs 35.4 %, and 97.8 vs 90.4 %, respectively). The difference was even higher for small-sized lymph nodes [97]. In another study, the performance of MRL at 3.0 T to detect LNM in patients with prostate and/or bladder cancer with no enlarged lymph nodes on previous CT or MRI was investigated. It was shown that MRL using quantification of signal-to-noise ratio (SNR) reduction could improve detection of LNM in normal-sized pelvic lymph nodes [98]. It has also been shown that MRL can detect LNM better than MDCT. In a study by Heesakkers et al. [99], it was reported that MRL could detect LNM with a higher sensitivity than MDCT (82 vs 34 %) in patients with intermediate or high risk of LNM (risk of >5 % according to nomograms). However, MDCT was slightly more specific than MRL (97 vs 93 %).

A combination of USPIO-enhanced MRI and DW-MRI was a promising method to detect LNM in normal-sized lymph nodes in a study by Thoeny et al. [100], who reported that this method was fast and reliable for nodal staging in patients with prostate and/or bladder cancer. The use of USPIO has been limited to investigational studies and has only been approved in some European countries, and to date has not been approved by the health authorities in the U.S.A. The production of the contrast agent has been discontinued and future clinical applications are unknown at this time.

Detection of Distant Metastasis

MRI can detect early bone marrow changes before osteoblastic changes appear in the bone marrow. MR appearance of bone metastases are signal loss which is in contrast to the high signal of surrounding marrow with fat on T1WI. The T2WI with fat suppression or short tau inversion recovery (STIR) are helpful to better visualize the bone metastases [22]. In a study by Lecouvet et al. [23] it was shown that MRI of discrepant or equivocal sites of bone lesions improved the bone metastases detection compared with BS combined with TXR, sensitivity 82 vs 63 % and specificity 100 vs 84 %, respectively. Figure 4.5 illustrates appearance of small bone metastasis on MRI, CT, and BS, and how the equivocal lesion on BS can be confirmed as benign by cross-sectional imaging.

DCE-MRI has been studied in various settings for tumor detection and particularly in recurrent tumors. Kayhan et al. [101] have investigated the enhancing pattern and quantitative values for bone metastases from prostate cancer and compared them with normal bone. Bone metastases showed significant enhancement and high permeability compared with normal bone. The normal bone was not enhancing in most of the cases. Authors concluded that high temporal resolution DCE-MRI can detect enhancing bone metastases in a background of non-enhancing normal bone. Figure 4.6 demonstrates an example of Axial DCE-MRI of a right femur bone metastasis.

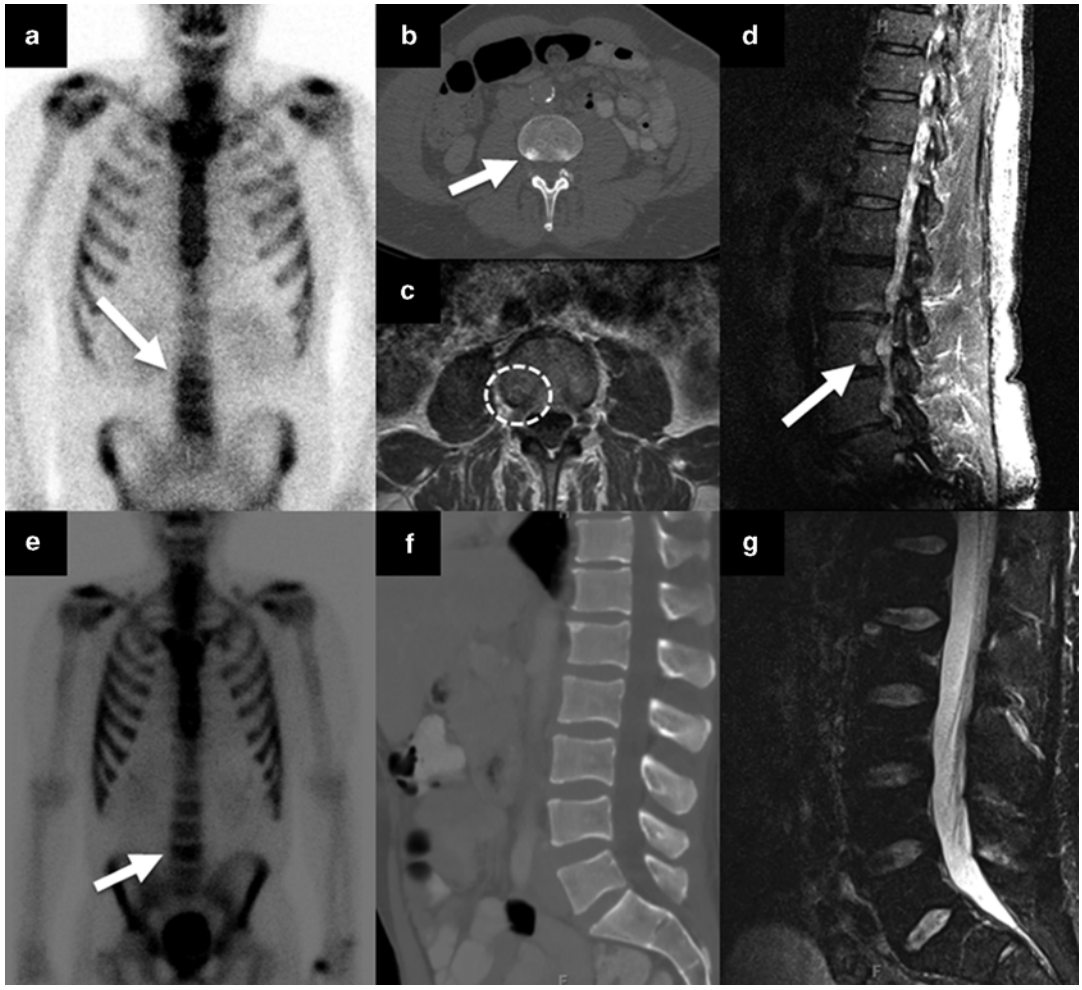

Fig. 4.5 Appearance of a bone metastasis and benign lesion on cross-sectional images and BS. A 67-year-old man with biopsy Gleason score 5+4, BS showed mild uptake on the right at the L3 vertebral body level (**a**), CT showed 1 cm rounded sclerotic focus on the right side of the L3 vertebral body (**b**), and confirmatory findings on MRI identified metastasis with focal signal loss combined with peripheral rim enhancement on T1WI (**c**) and high signal lesion on STIR sequence (**d**). A 53-year-old man with biopsy Gleason score 4+5, BS showed focal increased tracer uptake at L4 vertebral body level (**e**), CT revealed no focal lesion (**f**) with only minimal degenerative changes and likely lordotic strain responsible for the increased radiotracer uptake on E, as on MRI there was no abnormal signal on STIR sequence (**g**)

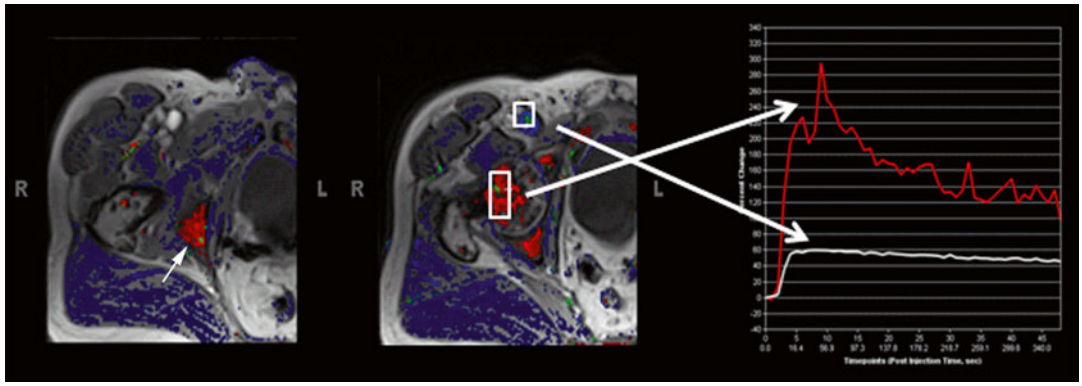

Fig. 4.6 Axial DCE-MRI of the right femur lesion shows abnormal perfusion (*red*) in the bone marrow (*arrow*) with washout kinetics characteristic for metastasis. Courtesy of Michael A. Jacobs, Ph.D. (JHU)

The axial skeleton MRI (AS-MRI) examines the presence of bone metastases for patients with high-risk prostate cancer in spine and pelvi-femoral region. Advances in MR techniques enable performance of whole-body MRI (WB-MRI) with the same field of view as BS and good spatial resolution. Therefore, all of the skeleton sites, including appendicular and axial skeleton, are examined by WB-MRI [102]. The performance of WB-MRI and AS-MRI has been compared in a cohort of 60 patients with prostate cancer and high risk for metastasis. It was shown that WB-MRI does not have an advantage over the AS-MRI for detection of bone metastasis [103]. Whole-body DW-MRI is an emerging advanced tool for at-a-glance assessment of the entire body in patients with cancer at high risk of distant metastasis. The whole-body DW-MRIs are best acquired at 1.5 T, the magnetic field strength at which the uniform fat suppression in the large whole-body field of view is performed best. Despite higher SNR achieved by higher magnetic field strength at 3.0 T, susceptibility artifacts and poorer fat suppression make the acquisition of whole-body DWI more challenging [104].

Recently, Lecouvet et al. [105] investigated the performance of WB-MRI and DWI in detection of metastasis in patients with prostate cancer at high-risk for metastasis and compared results with the performance of BS and CT. The sensitivity of WB-MRI was significantly higher than BS/TXR (98–100 % vs 86 %), while the specificity was similar (98–100 % vs 98 %), respectively. Two readers identified bone metastases in 7 and 8 of 55 patients with negative BS/TXR using WB-MRI. The sensitivity of WB-MRI was higher than the combination of BS/TXR and CT for detection of bone metastases and/or enlarged lymph nodes (91–94 % vs 84 %), while the specificity was similar (91–96 % vs 94–97 %), respectively. Authors concluded that WB-MRI including diffusion-weighted sequence is an excellent imaging modality to assess the disease spread in patients with prostate cancer at high-risk for metastasis as a one-step technique [105]. However, the cost-effectiveness and experience of readers are still crucial factors to consider. Figure 4.7 demonstrates diffusely metastatic bone metastases on WB-DWI. Figure 4.8 demonstrates detection of a left pelvic nodal metastasis on both FDG PET/CT and MRI WB-DWI.

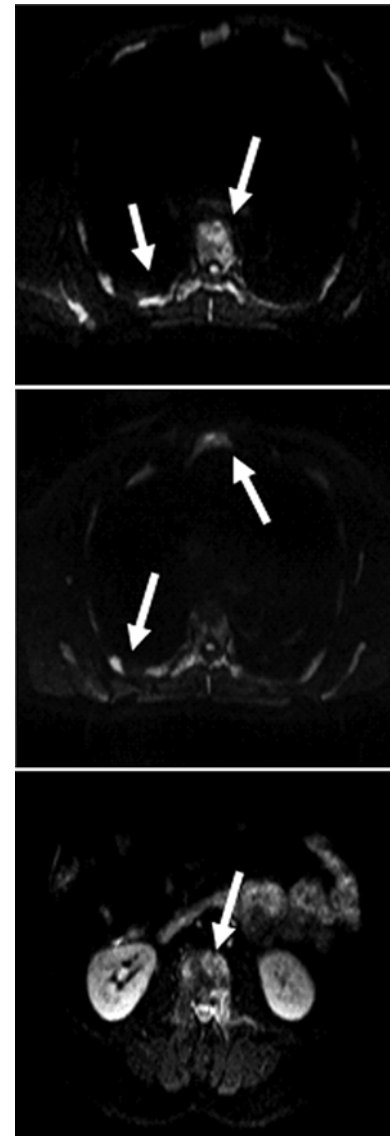

Fig. 4.7 A 64-year-old male with diffusely metastatic prostate cancer. Multiple foci of restricted diffusion on axial images from WB-DWI ($b=800$) within the ribs, sternum, and spine compatible with metastases (*arrows*). Courtesy of Michael A. Jacobs, Ph.D. (JHU)

In DWI, there are some motion-induced low SI foci on high *b*-value DWI which are considered as "blind spots" and make detection of metastases challenging. These areas are mediastinum, pulmonary hila, and most superior left

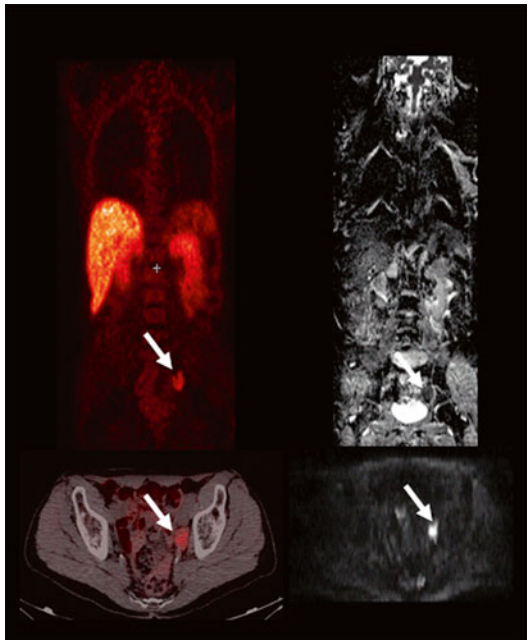

Fig. 4.8 A 78-year-old male with prostate cancer presents with rising PSA. FDG PET-CT (*left*) and WB-DWI (*right*) show left pelvic metastatic node (*arrow*) with avid FDG uptake (*left*) and corresponding restricted diffusion on ADC map in coronal plane and axial DWI $b = 800$ (*right*). Courtesy of Michael A. Jacobs, Ph.D. (JHU)

hepatic lobe just beneath the heart. Thus, metastatic bone lesions of the anterior chest wall are less obvious than lesions found in the spinal and paraspinal regions [104]. Other challenges in detection of bone metastasis are low levels of tumor infiltration, skull vault and skull base metastases (due to adjacent high SI of the brain), and metastases within hypercellular bone marrow [104]. Non-malignant causes of high SI on high *b*-value DWI are bone marrow edema secondary to fracture, degenerative changes, bone infarction, infection and hemangioma, isolated islands of red bone marrow within yellow marrow, and treated but inactive lesions showing a T2 shine-through effect. The ADC map and anatomical images are helpful to differentiate those false-positive findings [104].

The incidence of new soft-tissue involvement is low (1.9 %) in patients with CRPC and bone only metastasis. The use of routine interval CT scan has not been shown to be effective to monitor the development of new soft-tissue metastasis in such patients, at least in phase II clinical trials with time to progression of less than 8 months. It has been recommended that CT scan is indicated at the time of disease progression recognized by BS or PSA elevation, clinical signs or symptoms of new soft-tissue disease, and for patients who remain in trials for more than 8 months without progression [106].

Evaluation of Treatment Response with MRI

Patients with CRPC may have relatively low serum PSA level despite disease progression, making the assessment of treatment response by PSA decline less accurate [107]. Other measures such as imaging are required to assess the therapeutic response in this subtype of disease. The latest revised Response Evaluation Criteria in Solid Tumors (RECIST) guideline can be utilized for therapeutic response assessment of bone metastases with an osteolytic component on CT or MRI [108]. However, prostate cancer typically involves bone in the form of osteoblastic lesions which are considered nonmeasurable on RECIST guideline. Thus, treatment response cannot be evaluated for metastatic CRPC using RECIST guideline.

Subtle treatment-induced changes require quantitative assessment of bone lesions which is not usually achieved by BS despite high sensitivity for detection of bone metastases from prostate cancer [106]. Diffusion-weighted MRI (DW-MRI) has been found as a potential cancer biomarker to monitor treatment response and predict treatment outcome [109].

DW-MRI detects tumor lesions based on water content and water molecule movement within the tissue. However, some microscopic features affect the water diffusion such as cell density, nuclear-to-cytoplasmic ratio, distribution of cell sizes within tissue, extracellular space tortuosity, integrity of cellular membranes, tissue organization (e.g., glandular formation), and tissue perfusion. These features are also involved in therapeutic effects [110].

Therefore, the DW-MRI appearance of soft tissue response to treatment is biphasic and

heterogeneous within tumor. There is transient increase in ADC once the tumor cells die, which is followed by decrease in ADC value when macrophages remove the dead cells and tissue remodeling happens. However, resistance to treatment is also associated with low ADC values and limits the application of this method to assess soft tissue response to treatment. In addition, radiation therapy causes prolonged, persistent high ADC value owing to tissue edema secondary to increased microvascular permeability, and tissue inflammation in normal radiated soft tissue [110].

Given the high number of fat cells and low water content, normal bone marrow exhibits low SI on high *b*-value images and low ADC values. The tumor replacement of bone marrow causes an increase in bone marrow cellularity and water content appearing as initial increases in SI on high *b*-value and ADC values. When the metastatic bone involvement progresses and fully replaces the fat cells, SI on high *b*-value images persistently increase while the corresponding ADC values decrease. The inconsistent changes in SI and ADC values for bone marrow in response to treatment compared with soft tissue response make the interpretation of changes of diffusion parameters for bone metastases more challenging [110].

Functional diffusion map (DM) has been recently utilized in small sample of patients to monitor treatment response. Given the heterogeneous treatment response within tumors, this method demonstrates the change in ADC values compared with pre-therapy ADC values on a per-voxel basis. Reischauer et al. [106] reported the result of a preliminary study on ADC changes using functional DMs in patients with pelvic bone metastases from prostate cancer with anti-androgen treatment. Treatment response was detected as significant increase in the mean ADC values of bone lesions as early 1 month after the initiation of treatment which was persistent at 3 months after treatment. However, functional DMs also showed increase of tumor volume with significantly decreased ADC values compared with pre-therapy ADC values reflecting the heterogeneous response to treatment within each individual tumor.

Conclusion

With growing therapeutics options for the treatment of metastatic and advanced prostate cancer, improved functional imaging of prostate cancer beyond the limitations of conventional computed tomography (CT) and bone scan (BS) is becoming increasingly important for both clinical management and drug development. Various current and promising emerging PET radiotracers beyond FDG PET/CT are being evaluated and will be applied in the CRPC setting as non-invasive imaging biomarkers. Current mMRI techniques, in particular diffusion-weighted MRI (DW-MRI) have the potential to monitor treatment response and predict treatment outcome in CRPC. The advent of multi-modality PET/MRI will help to synergize these two modalities to improve our understanding and management of men with metastatic prostate cancer.

Acknowledgments ^{18}F-DCFBC PET/CT images are unpublished research scans supported through the Prostate Cancer Foundation (PCF) Young Investigator Award. Michael A. Jacobs, Ph.D., for providing relevant MRI images.

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Part II

Treatment: The Androgen Receptor Signaling Axis Biology and Therapeutic Opportunities, Systemic Chemotherapy, Immunotherapy, Bone Targeted Approaches and Radiopharmaceuticals

Androgen Receptor Biology in Castration Resistant Prostate Cancer

5

Heather H. Cheng and Bruce Montgomery

Androgen Receptor Structure and Function in Prostate Cancer

Prostate cancer is the most common solid tumor and the second most common cause of cancer death in men in the USA, with over 29,000 men anticipated to die of metastatic disease in 2013 [1]. The role of androgens and the androgen receptor (AR) in prostate cancer was originally suggested by their critical role in normal development and maintenance of the prostate gland. Huggins and Hodges postulated that androgens and their receptors were essential targets and established the primacy of this axis by demonstrating that androgen suppression could effectively control prostate cancer progression [2]. The human AR is a nuclear receptor transcription factor located on chromosome Xq11-12, is structurally similar to other steroid hormone receptors, and is divided into distinct functional regions. These include the amino-terminal domain (NTD), DNA-binding domain (DBD), hinge-region (HR), and carboxy-terminal ligand-binding domain (LBD) (Fig. 5.1). The NTD is an activation function (AF) domain involved in co-activator binding, also called AF-1. It is also the least conserved region of the AR and contains poly-glutamine, poly-proline, and poly-glycine regions, ranging in length from 18 to 22 repeats (normal) to over 40 repeats (linked to non-malignant disease such as spinal and bulbar muscular atrophy) [3]. Due to a high degree of intrinsic disorder, the NTD has been difficult to crystallize and thus develop structure-based drug antagonists. The DBD mediates the critical interaction with androgen response elements (ARE) on DNA and is comprised of two zinc finger motifs. The first zinc finger defines DNA binding specificity, whereas the second zinc finger facilitates receptor dimerization and stabilization of the DNA-receptor complex. The HR contains a nuclear localization signal that is unmasked following ligand binding, change in receptor conformation, and release of chaperone proteins. The C-terminal LBD mediates receptor dimerization as well as ligand-dependent co-activator binding (AF-2).

In the absence of ligand, the AR is primarily located in the cytoplasm and bound to chaperone or heat shock proteins (such as HSP-90) which maintain receptor conformation and prevent degradation. The AR is activated by interaction with multiple steroid hormones, including testosterone (T), dihydrotesterone (DHT), and adrenal androgens, though the latter interaction occurs with significantly lower affinity. Binding of steroid

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F. Saad and M.A. Eisenberger (eds.), *Management of Castration Resistant Prostate Cancer*, Current Clinical Urology, DOI 10.1007/978-1-4939-1176-9_5,

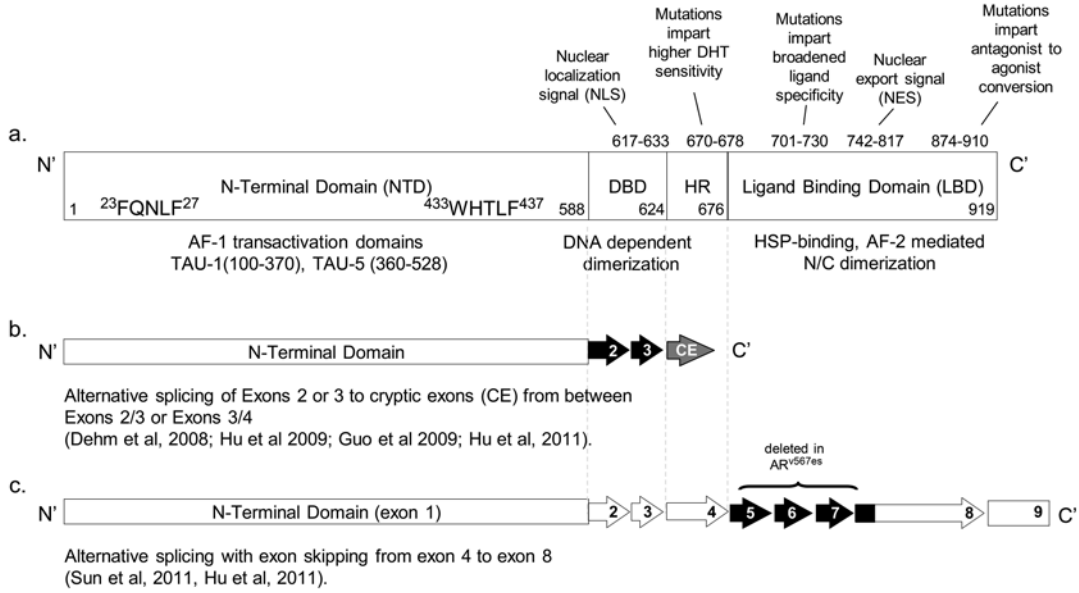

Fig. 5.1 Schematic of the full-length androgen receptor and exon structure of major splice variants (ARV7 (b) and ARV567 (c)). Domains of AR include the amino (N) terminal domain, the DNA-binding domain (DBD), the hinge-region (HR), and the carboxy (C)-terminal ligand-binding domain (LBD). Mutations which occur in specific regions are indicated (modified from Mostaghel et al. [70])

releases receptor chaperones and leads to conformational activation, receptor dimerization, phosphorylation, and nuclear translocation. The dimerized AR then binds to target DNA sequences (AREs) in promotor and enhancer regions of androgen-responsive genes, leading to selective recruitment of coregulator proteins and closely regulated activation or repression of gene expression.

Activation of the AR Axis in Castration Resistant Prostate Cancer

Like other hormones, AR signaling can mediate pleiotropic effects through affecting a broad array of genes that regulate cell cycle, survival, and proliferation [4–7]. Interference with androgen–AR interaction, such as by serum androgen suppression (androgen deprivation therapy), eliminates these signals and induces nearly universal responses in the treatment of prostate cancer as measured by declines in PSA and control of symptoms in symptomatic patients. Early events after initiating blockade of AR signaling (days 0–3) include downregulation of AR and expression of the negative cell cycle regulators, p21 and p27. Apoptosis resulting from AR signaling blockade ranges from 0 to 20 % within the first 7 days depending on the method of assay and the tissue evaluated [8, 9]. Apoptosis after androgen deprivation in men treated with castration increases within the first 24 h, with a maximum effect (2.5–3 % apoptosis) at 3–4 days with subsequent declines in the numbers of apoptotic cells to baseline over the following week [10, 11]. Subsequently (following day 3), the proliferation rate begins to decrease (as measured by Ki67 staining) and is associated with increase in cell cycle arrest in prostate cancer xenografts.

Despite initial responses to AR signaling blockade, the majority of cancers become resistant to androgen deprivation using the currently available agents. This disease state was initially considered a "hormone refractory" phenotype as most patients maintained anorchid serum testosterone levels. However, intra-tumoral AR signaling

continues to drive cancer progression despite low testosterone blood levels, therefore this state is now considered "castration resistant" prostate cancer (CRPC). The increase in serum PSA in the context of clinical progression serves as compelling evidence supporting the importance of AR signaling in CRPC since PSA is directly and exclusively driven through AR-mediated transcription. Molecular analysis of tumor tissue demonstrates that the majority of the AR transcriptional program is reactivated in progressive CRPC [12]. Intra-tumor androgen levels in metastases from CRPC patients exceed intratumor androgen levels in primary prostate tumors from treatment naïve patients [13]. Sources of intra-tumor androgens outside the testes potentially include circulating adrenal androgens, as well as intracrine androgens synthesized de novo within prostate cancer cells [13–15].

Adaptation to the CRPC state also involves substantial upregulation of AR expression; not only by amplification of the AR locus (as seen in 20–30 % of CRPC tumors), but also by increased transcription and stabilization of mRNA or protein [16]. Increased AR expression serves to overcome castrate androgen levels, and has been shown to be both necessary and sufficient in inducing tumor growth in prostate cancer models [17].

Strong confirmatory evidence that both tissue androgens and AR itself are drivers of CRPC comes from the phase III studies of next generation agents targeting these two arms of AR biology. Abiraterone is a selective inhibitor of the steroidogenic enzyme CYP17 and suppresses both serum and tissue androgen levels more effectively than standard androgen deprivation [18–20]. This agent, combined with LHRH agonists, provided survival and quality of life benefits in both chemotherapy-naïve and post-docetaxel CRPC populations, leading to FDA approval in both settings and confirming that androgens drive disease progression in CRPC [21, 22].

Enzalutamide (formerly MDV3100) is a competitive AR antagonist that binds AR with 5–8 fold greater affinity than earlier anti-androgens. In the phase III randomized (AFFIRM) study, enzalutamide improved overall survival by 37 % compared to placebo in men with CRPC previously treated with docetaxel [23], again confirming that therapy directly targeting AR provides clinical benefit in CRPC [24].

AR-Specific Adaptation in CRPC

The selective pressures of androgen deprivation and AR antagonism lead to mutation and aberrant transcription of AR that minimize or eliminate the need for DHT and testosterone. AR mutations were initially identified in the LBD in a high proportion of cancer from patients with CRPC [25], suggesting these mutations as primary drivers of resistance to hormone therapy. Subsequently, multiple classes of AR mutations have been identified in additional regions of the receptor, which result in broadening of ligand specificity and/or conversion of antagonists to agonists (Fig. 5.1) [26, 27]. Evidence also suggests that AR mutations are more prevalent in patients previously treated with first generation AR antagonists such as flutamide and bicalutamide [28]. The frequency of AR mutation in CRPC tumors treated with LHRH agonist or orchiectomy alone is low (8–25 %), suggesting that anti-androgen exposure drives mutation, but that mutation does not a primary driver resistance to ADT alone [32, 28]. More recent studies are using whole exome sequencing of metastatic CRPC in patients before and after treatment with abiraterone and enzalutamide to describe the presence of AR mutations. These studies may reveal that AR mutation becomes a more critical mechanism of secondary resistance under the selection pressure of more effective AR axis blockade. Hints that this may be the case come from several studies documenting a novel AR mutation (F876L) generated by in vitro selection with enzalutamide. AR_{F876L} renders the next generation AR antagonists, enzalutamide and ARN-509, into AR agonists [29–31]. AR_{F876L} have been detected in plasma cell-free DNA from patients progressing on enzalutamide and ARN-509, suggesting that this mutation may be clinically relevant. Further interrogation of ARF876L from patients progressing on abiraterone and enzalutamide, including in metastases, will determine the degree to which

AR_{F876L} and other AR mutant forms affect resistance in the context of more complete suppression of AR signaling.

An alternative AR-mediated mechanism of resistance to androgen deprivation is the induction of alternative pre-mRNA splicing to generate constitutively active AR species. During pre-mRNA alternative splicing, aberrant combinations of coding (exons) and non-coding (introns) regions of pre-mRNA transcripts from a single gene and are translated into protein isoforms with differing biological functions. Recent evidence suggests that aberrant AR splicing results in AR variants (ARVs) lacking the LBD and expressing ligand-independent constitutive activity [32–37] (Fig. 5.1). These ARVs can homodimerize or heterodimerize with full-length AR and initiate AR signaling in the absence of ligand [36]. Although a large number of ARVs have been described from cell lines [38], only ARV7 and ARV567 variants have been commonly identified in human CRPC tumors [32, 34, 36]. ARV7 and ARV567 exhibit both unique and overlapping transcriptional programs compared to wild-type AR [39]. While ARVs lacking the LBD drive ligand-independent growth when evaluated in prostate cancer models, the precise role of ARVs in prostate cancer development and progression remains controversial. However, a number of findings suggest that ARVs play a role in prostate cancer pathogenesis: First, ARV7 can be identified in normal prostate epithelium and is associated with a shorter time to recurrence after prostatectomy [34, 35]. Second, murine transgenic expression of ARV567 in prostate epithelium leads to adenocarcinoma by 50 weeks (S. Plymate, *in preparation*). Finally, increased levels of ARV7 and ARV567 were associated with shorter survival in patients with CRPC and bone metastases [32, 34, 35].

A recent debate has focused on whether the presence of ARVs is relevant given that the relative proportion of ARVs vs. wild-type AR in experimental settings, with splice variants found at levels a log lower than wild-type. It is important to note that a subgroup of bone metastases demonstrated nearly equivalent protein levels of full-length and truncated ARVs by western blot [32].

The induction of ARVs following castration may be an important step in prostate tumor survival, or may provide a bridge to induction of additional tumor growth mechanisms [40]. Additional controversy arises regarding whether outgrowth of ligand-independent tumor cell clones ARVs are generated primarily through aberrant splicing or through genomic rearrangement of AR [41].

Adaptation in AR-Regulated Pathways

Recent work suggests that the mechanism by which AR undergoes nuclear translocation occurs through binding of a canonical nuclear localization signal in exon 4 within AR to cellular microtubules at dynein motors [42, 43]. Although the activity of taxanes in other malignancies is primarily ascribed to microtubule stabilization, it has been proposed that this unlikely to be the primary mechanism of action in prostate cancer due to its relatively slow proliferation [44]. In a recently proposed model, taxanes inhibit prostate cancer growth not only through G2-M arrest, but also through inhibition of AR translocation to the nucleus. This model is supported by the correlation of cytoplasmic sequestration of AR in circulating tumor cells (CTCs) with clinical response to taxane therapy [44].

Importantly, taxane-mediated nuclear exclusion may affect ARVs differently compared to wild-type AR. The nuclear localization signal of ARV7 is located in the cryptic exon and is distinct from that of wild-type AR and ARV567 [45]. As a result, ARV7 is resistant to taxane-mediated nuclear exclusion, potentially abrogating taxane efficacy [46]. This may be clinically relevant since not only does preclinical data demonstrate that ARVs contribute to resistance to abiraterone and enzalutamide, but emerging clinical data also suggests patients with tumors resistant to abiraterone and enzalutamide are less sensitive to docetaxel [47]. This implies that ARV expression may regulate sensitivity to both AR targeting agents and taxanes, and, further suggests that targeting the NTD may enhance taxane efficacy in ARV-expressing tumors.

Nuclear Receptor Superfamily Crosstalk in AR Signaling

Androgen deprivation exerts selection pressure to preserve AR signaling in two ways: First, it can broaden AR ligand specificity to include ligands of the closely related steroid receptor superfamily (e.g., progesterone, cortisol). Second, other members of the nuclear receptor superfamily (e.g., glucocorticoid receptor (GR), mineralocorticoid receptor and progesterone receptor) can be recruited to maintain AR signaling. These receptors have strong sequence homology in the DBD to AR, and may maintain AR signaling in the androgen-deprived state by allowing access to transcription factor binding sites in AR-regulated genes. For example, the GR shares transcriptional response elements with AR in multiple gene targets, and activates a transcriptional program largely overlapping with that activated by AR [48]. The FOXA1 transcription factor regulates differential binding of GR and AR to gene targets, and thereby regulates GR function in prostate cancer [48]. The strongest preclinical and clinical evidence to support a role for GR in castration resistance comes from a study characterizing prostate cancer cell lines selected for resistance to ARN-509 and enzalutamide [49]. Glucocorticoid receptor was markedly upregulated as measured by expression array analysis and confirmed by Western blot, and GR knockdown partially abrogated the enzalutamide-resistant phenotype when cell lines were grown as xenografts [50]. Moreover, AR and GR share largely overlapping cistromes and transcriptional programs. Evaluation of bone metastases in patients treated with enzalutamide has demonstrated increased expression of GR in enzalutamide-resistant metastases compared to enzalutamide-responsive metastases.

A post-hoc analysis of the phase III AFFIRM trial (a study of enzalutamide in patients previously treated with docetaxel) suggested that use of glucocorticoids was associated with inferior survival (independent of other known prognostic factors) and could be a factor in driving adverse biology [51]. A similar analysis of the phase III COU-301 trial (a study of abiraterone in patients previously treated with docetaxel) suggested that use of glucocorticoids was associated with greater comorbidity and worse disease outcomes [52]. A more thorough understanding of how GR biology drives progression in CRPC and the impact of glucocorticoid use in patients with CRPC will require additional clinical and translational studies.

Growth Factor Crosstalk in AR Signaling

A number of signaling pathways, including receptor/nonreceptor tyrosine kinases and cytokines, have been proposed to mediate ligand-dependent and independent AR activation. The proposed mechanism is that activation of tyrosine kinases or G-protein coupled receptors then activates AR transcriptional programs through alteration of the AR itself through phosphorylation or through modulation of components of the AR coregulator complex.

The cytokine IL-6 is produced by multiple cell types, including CRPC, and is a strong negative factor for patients with CRPC [53]. IL-6 induces the AR coregulator p300 and activates AR transcriptional programs in an androgen-independent manner [54, 55]. To date, targeting of IL-6 with monoclonal antibodies alone, or in combination with chemotherapy, has failed to provide clinical benefits for patients with CRPC [53, 56].

Many kinase pathways have been proposed to modulate AR activity by phosphorylation, including SRC, EGFR, ELK, HER2, and IGF-1R [57–61]. Androgen receptor phosphorylation modulates stability, nuclear translocation, and binding to relevant regulatory elements [57, 62]. Targeting a specific kinase pathway has been challenging given the number of potential redundancies between pathways. To date, clinical studies targeting these kinases, including EGFR, HER2 and SRC, have failed to identify effective agents, leaving this approach attractive but unproven [63–65].

Modulation of AR Co-regulatory Networks

Following AR translocation from the cytoplasm into the nucleus, the dimeric receptor binds to androgen response elements (ARE) on DNA and

recruits co-regulatory proteins (including transcriptional coactivators and corepressors) to modulate transcription of target genes. The resulting protein complexes may change AR transcription by modulating chromatin structure and interacting with the RNA polymerase transcription complex.

AR is distinguished from other nuclear steroid receptors in that many of its binding sites do not contain the canonical nuclear receptor-binding motif, and may contain only a fraction of the ARE [66]. This suggests that co-regulatory complex formation, including other transcription factors, plays a more critical role in AR target gene transcription compared to other nuclear steroid receptors. Mapping of AR transcription factor binding sites in castration sensitive and CRPC tumors demonstrates they have distinctly different transcriptionally active sites, particularly with respect to genes regulating mitosis and cell cycle [5].

FOXA1 is a member of the forkhead family of transcription factors and has been proposed to play a critical role in CRPC. Members of this family are called "pioneer factors" because of their role in relaxing condensed chromatin and initiating recruitment of transcription factors to relevant binding sites. FOXA1 recruits multiple nuclear receptors to DNA binding sites, and is therefore expected to facilitate AR binding in prostate cancer cells. However, the role of FOXA1 may be more nuanced, as silencing of FOXA1 resulted in marked redistribution of AR binding sites in chromatin [48]. These findings strongly suggest that FOXA1 not only facilitates access of AR to some binding sites, but also masks other binding sites. Subsequent work suggests that FOXA1 regulates the considerably overlapping transcriptional programs of both AR and GR, and thus may have an important role in mediating anti-androgen resistance via GR signaling [49, 67].

p300 is a histone acetyl transferase and, together with IL-6, coactivates AR transcription [55], therefore has also been proposed as a critical coregulator in CRPC progression. Androgen deprivation substantially increases p300 levels, though the presence of a functional AR pathway is required for p300 modulation [68]. Knockdown of p300 decreased proliferation by suppressing cyclin proteins critical for cell cycle progression and suggests that under androgen-deprived conditions, p300 is upregulated to maintain AR signaling and cell survival.

Summary

The AR axis remains the central target in treating the most lethal form of prostate cancer, a conclusion supported by several phase III studies documenting clinical benefit from more potent AR blockade. Although most solid tumors are, by nature, heterogeneous, the majority of CRPC appears to be one of the two phenotypes: (1) "intracrine-dependent" tumors, which are those capable of activating AR through elaboration of tumoral androgens and (2) "ligand-independent" tumors, which remain dependent on aberrant AR signaling, including AR crosstalk with other pathways. The mechanisms of resistance to our current most effective androgen blocking agents, abiraterone and enzalutamide, continue to drive an AR transcriptional program [20, 31, 49, 69]. The challenge now is to determine to what degree aberrant AR isoforms, breakthrough androgen synthesis, or parallel signaling through other nuclear receptors are clinically relevant resistance mechanisms and how they can be effectively targeted. Until the AR-axis has been effectively extinguished, we will be unable to answer the ultimate question of whether AR targeting can cure both early- and late-stage prostate cancer.

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6 The Androgen Receptor as a Therapeutic Target for Castration-Resistant Prostate Cancer

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The Androgen Receptor

The androgen receptor (AR) is the molecular engine responsible for prostate cancer growth and survival over the entire course of the disease, from a patient's diagnosis to his death from metastatic disease. While previous conceptualizations of advanced prostate cancer postulated that the disease became "hormone refractory" or "androgen independent" after treatment to reduce serum testosterone, the current framework for the treated history of prostate cancer recognizes that the AR continues to signal even after serum testosterone is suppressed to castrate levels. Hence, the contemporary model of advanced prostate cancer incorporates an active, functional AR that continues to signal long after serum testosterone levels are reduced, so that the tumor remains susceptible to further hormonal manipulation. Such advances in our understanding of the biology of castration-resistant prostate cancer (CRPC) have led to the development of novel therapies that confer tangible clinical benefits to patients, even in the terminal phases of the disease.

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The biology of the AR, which underlies current anti-AR strategies, is complex, and our understanding of it is rapidly expanding. To adequately appreciate the underlying science behind clinical strategies to target the AR molecule, a summary of this biology is essential. Located on chromosome Xq11-12, the AR is a 110 kDa steroid receptor in the superfamily of nuclear receptors [1]. It is found on benign prostate epithelial cells as well as prostate cancer cells at all stages and grades. It is a ligand-activated transcription factor that contains three distinct domains: a C-terminal ligand-binding domain (LBD; exons 4–8), which regulates ligand-dependent activation; an N-terminal domain (NTD; exon 1), which is essential for transcriptional activity; and a DNA-binding domain (DBD; exons 2–3). A hinge region between the DBD and the LBD regulates the mechanisms underlying protein degradation and nuclear localization [2–7]. In its inactive or unbound form in the cytoplasm, the AR is an unstable protein in a complex with heat-shock proteins (Hsp), particularly Hsp90, and other co-chaperones (Fig. 6.1). On activation, or when bound to the ligand, the AR dissociates from the Hsp, resulting in dimerization and nuclear translocation, followed by binding to specific DNA sequences known as androgen-responsive elements. Posttranslational modification of the AR by phosphorylation, methylation, acetylation, ubiquitination, and sumoylation help stabilize the AR and assist in cellular localization and transcriptional activity [8]. Through interaction with the AR's NTD and LBD, transcriptional

F. Saad and M.A. Eisenberger (eds.), *Management of Castration Resistant Prostate Cancer*, Current Clinical Urology, DOI 10.1007/978-1-4939-1176-9_6,

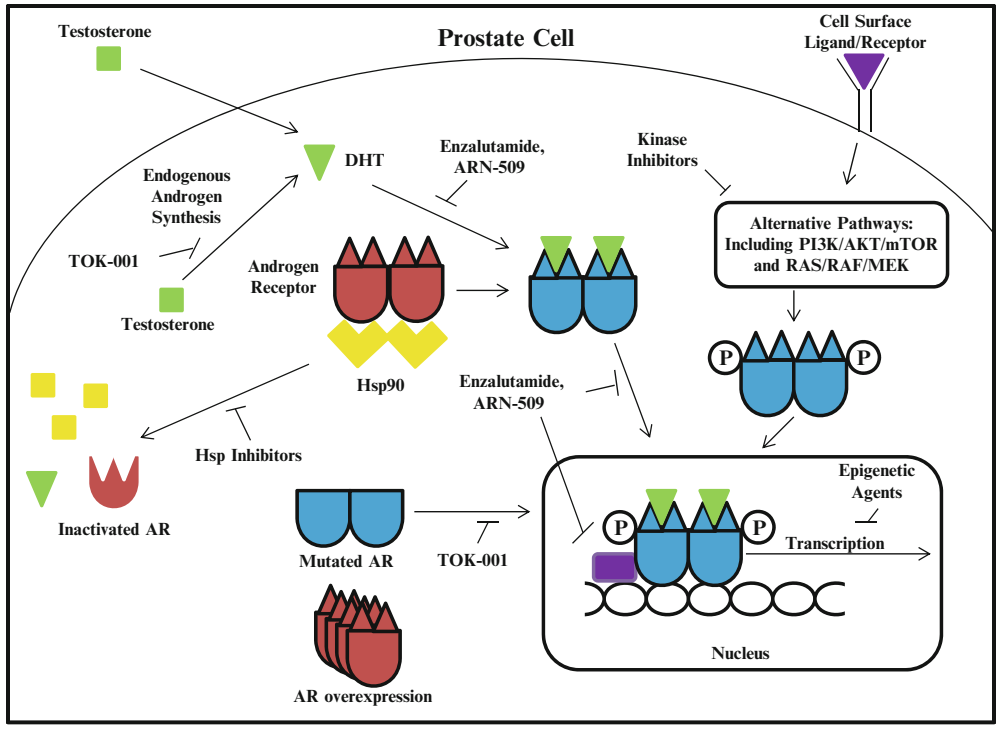

Fig. 6.1 The androgen receptor pathway and novel targeted agents

coactivators assist in the transcription of numerous AR-regulated genes, including prostate-specific antigen (PSA) [6, 9].

The activated AR can also function as a transcription repressor through its antagonistic interaction with transcription factors (e.g., specificity protein 1 [SP1] and Sma and Mad related protein-3 [SMAD3]) and its direct recruitment of transcriptional repressors (e.g., Alien and small heterodimer partner [SHP]) [10]. This dual functionality has important implications in terms of the receptor's response to androgen deprivation therapy (ADT), currently the standard treatment for metastatic CRPC. Androgen depletion initially leads to an interruption of the AR signaling pathway, but the prostate cancer cell has developed multiple mechanisms to adapt to this deprivation and regain its metabolic function. Thus, a better understanding of the basic mechanisms and function of the AR-ligand pathway, as well as its ability to adapt to a clinical castrate state, has led to recent advances in drug discovery and resulted in improved outcomes in this patient population.

AR Signaling in CRPC

As patients undergo castrating therapy, their tumors undergo adaptive changes by which the AR molecule retains its role as the primary engine of tumor growth despite treatment. AR overexpression [11], mutations within the AR, intracrine synthesis of androgen [12, 13], AR splice variants [14, 15], and activation of alternative pathways such as the phosphatidylinositol-3-OH kinase (PI3K)/AKT/mammalian target of rapamycin (mTOR) pathway [16] are some of the means by which the AR continues to signal in the castrate state.

AR gene amplification and protein overexpression are thought to be two of the main mechanisms driving CRPC. In xenograft models, AR overexpression was found to be both necessary and sufficient to induce CRPC [17]. In addition, studies utilizing expression profiling analysis and fluorescence in situ hybridization have identified AR amplification and protein overexpression in approximately 30 % of recurrent prostate cancer

specimens [18–20]. A number of androgen-related genes have been found to be upregulated after ADT; this process is thought to lead to sensitization of the tumor cell to lower levels of androgens and reactivation of the AR-ligand pathway thus allowing CRPC to proliferate in an androgen depleted environment [11, 13, 19, 21–25]. Furthermore, overexpression of the AR has also been found to be a potential driver of first-generation antiandrogen agonist activity [17].

Mutations within the LBD and NTD of the AR have also been identified as mechanisms of resistance in CRPC. Specifically, mutations within the LBD can confer resistance to antiandrogen therapy and allow for decreased specificity of AR–ligand interaction, thus enabling alternative steroidal molecules (e.g., estrogens, corticosteroids, and progesterone) to activate the AR pathway [26, 27]. Additionally, molecular dissection of the NTD transactivation unit 5 domain has demonstrated that a WxxLF motif was fully responsible for ligand-independent activity [28].

The recognition of AR splice variants in tissue from patients with CRPC has provided more insight into the resistance mechanisms driving this disease [29, 30]. Although AR splice variants lack an LBD, they contain an intact NTD and DBD and have been shown to be active in the absence of androgens [31, 32]. Regulation of AR splice variants remains poorly understood; however, increased expression has been seen in prostate cancer cell lines that have been introduced to an androgen antagonist [33]. At least seven splice variants of the AR have been uncovered to date, and two of these variants have up to a 20-fold higher expression in CRPC compared to castration-sensitive prostate cancer; and higher expression of one variant, AR-V7, was seen to predict biochemical recurrence following definitive local therapy in castration-sensitive disease [34].

Finally, kinase pathway activation is yet another mechanism central to the development of CRPC. The PI3K/AKT/mTOR pathway plays an important role in tumorigenesis and therapy resistance in multiple malignancies, including prostate cancer, and activation of this pathway is strongly associated with prostate cancer progression [35–37]. PI3K/AKT/mTOR pathway alterations are seen in up to 43 % of primary prostate cancer cases and up to 100 % in metastatic disease, with loss of *PTEN* (phosphatase and tensin homolog), a tumor suppressor gene, accounting for roughly 40 % of cases [35, 37]. Loss of *PTEN* has been correlated with resistance to castration in in vitro models, though this effect is not absolute [38, 39].

All of these mechanisms—gene amplification, protein overexpression, constitutive activation, and promiscuous mutation—are mechanisms of resistance in CRPC. All of these, as well, point to the AR as a prime target for treating CRPC to yield true clinical benefits.

First-Generation Antiandrogens

First-generation nonsteroidal antiandrogens include flutamide, nilutamide, and bicalutamide. These nonsteroidal agents compete with endogenous androgens for binding within the LBD, resulting in a conformational change in the AR that inhibits transcriptional activity [40].

Clinically, these drugs have traditionally been used to prevent flare, which is seen in the setting of initial gonadotropin-releasing hormone (GnRH) use as well as in combined androgen blockade (CAB) therapy. Flare occurs in roughly 10 % of patients starting GnRH agonist therapy and is related to the surge of androgens during the first week of treatment; this surge is potentially responsible for a rise in PSA and worsening of clinical symptoms [41]. The flare phenomena in patients with advanced disease can lead to worsening back pain, urinary obstruction, and other negative symptoms, and is routinely suppressed with the use of concurrent administration of a GnRH analog and an antiandrogen agent. These drugs have also been used chronically in conjunction with surgical or medical castration as part of CAB therapy, a controversial strategy whose benefits remain ambiguous. At least three large meta-analyses evaluating the survival advantage of CAB have been published. One analysis showed a 10 % improvement in overall survival after CAB (relative risk = 0.90; 95 % confidence interval [CI] 0.79–1.00), another

revealed no survival advantage, and the third found no benefit at 2 years (20 trials, hazard ratio [HR] = 0.970; 95 % CI 0.866–1.087) but a statistically significant difference in survival at 5 years (10 trials, HR = 0.871; 95 % CI 0.805–0.942) [42–44]. Therefore, CAB therapy might potentially benefit a subset of patients, although the relative effect on all patients with prostate cancer remains uncertain.

Whether antiandrogens are used for flare prevention or as a component of CAB, most patients who initially respond to ADT eventually progress to CRPC via the mechanisms described above. Specific mutations within the AR have also explained tumor growth in the setting of antiandrogen use [45, 46]. For example, a novel mutation within codon 741 of the AR LBD has been shown to convert bicalutamide from an antagonist to an agonist, while flutamide acquires agonistic properties when exposed to mutations on codons 874 and 877 [47]. As a result, a subclass of patients with these mutations will develop a paradoxical decline in their PSA level and tumor size when conventional antiandrogens are withdrawn, indicating an agonistic property [48–50]. Specifically, a Southwest Oncology Group clinical trial (SWOG 9426) examined the antiandrogen withdrawal phenomenon using a variety of first-generation antiandrogens and found that 21 % of the 210 patients enrolled had a confirmed PSA decline of ≥50 % at the termination of antiandrogen use. Of these patients, 64 % were on flutamide, 32 % on bicalutamide, and 3 % on nilutamide [48]. A phase 3 clinical trial, CALGB 9583, compared patients undergoing antiandrogen withdrawal either alone or in combination with ketoconazole. That study found that 15 of 132 patients (11 %) of patients who underwent antiandrogen withdrawal alone demonstrated a PSA decline of ≥50 %, with a median time to PSA progression of 5.9 months [51]. The antiandrogen withdrawal phenomenon highlights the inability of first-generation antiandrogens to durably repress AR signaling, as they can serve as partial agonists despite initial repression of signaling. This key weakness spurred a search for new drugs that did not have partial agonistic activity and thus repressed the AR more fully and more durably.

BMS-641988

BMS-641988 is a competitive inhibitor of the AR found to partially overcome some of the pitfalls of first-generation antiandrogen agents. In preclinical studies using the CWR-22-BMSLD1 human prostate cancer xenograft model, BMS-641988 displayed an increased efficacy over bicalutamide, with an average tumor growth inhibition of >90 % versus <50 %, respectively. In addition, researchers administered BMS-641988 to a bicalutamide-refractory CWR-22-BMSLD1 model and found that BMS-641988 significantly delayed tumor growth, whereas the group that continued bicalutamide therapy had tumors that grew progressively. However, once BMS-641988 therapy was stopped, tumors in that group restored their capacity to grow reflecting a more cytostatic rather than cytocidal effect [52]. Its ability to bind to the wild-type AR with a 20-fold higher affinity than bicalutamide and display activity towards the mutant AR eventually led to BMS-641988 to be studied in a clinical trial setting.

BMS-641988 was investigated in a phase I dose-escalation study involving 61 men with metastatic CRPC (Table 6.1). A majority of patients enrolled on the trial (65 %) had undergone three or more treatment regimens, including 16 (26 %) who had received prior chemotherapy with docetaxel. A dose range between 5 and 140 mg per day was evaluated, with no patients remaining on therapy at the completion of the trial. The two most common adverse events were fatigue (25 %) and gastrointestinal events (31 %), all of which were grade 1 or 2. However, one patient had a grade 3 seizure event while receiving 60 mg of BMS-641988, which resulted in termination of the trial. At the completion of the trial, 10 of the 61 patients (16 %) had a >30 % decline in PSA. Of 23 patients with measurable disease, one (4 %) had a partial response and 17 (74 %) had stable disease on imaging. In addition, after termination of treatment with BMS-641988, some patients had a decline in their PSA, revealing a possible antiandrogen withdrawal response and implying that BMS-641988 might have both agonistic and antagonistic properties. Therefore, as a result of its serious adverse event

Table 6.1 Clinical trials of androgen receptor targeted agents for CRPC

Class	Agent	Phase	Treatment arms	Primary end point	Remarks and patient population	Reference/ClinicalTrials.gov identifier
Targeted agents	Enzalutamide (MDV3100)	III	Double-blinded placebo controlled: enzalutamide vs. placebo	OS	AFFIRM trial, post-docetaxel-based chemo mCRPC. Trial stopped early due to benefit	[65], NCT00974311
	Enzalutamide (MDV3100)	III	Double-blinded placebo controlled: enzalutamide vs. placebo	OS and rPFS	PREVAIL trial, pre-chemo mCRPC. Trial stopped early due to benefit	[66], NCT01212991
	BMS-641988	I	Randomized dose-escalation	Safety and tolerability	Study closed due to limited antitumor affect and seizure activity	[53]
	ARN-509	I/II	Non-randomized, open label	PSA response	Three arm: treatment-naïve nonmetastatic CRPC vs. treatment naïve mCRPC vs. post-abiraterone mCRPC	[77], NCT01171898
	ARN-509	Ib	Open label: ARN-509 + abiraterone + prednisone	MTD/RP2D when administered with abiraterone	mCRPC	NCT01792687
	ARN-509	II	Randomized, open label, three arm: ARN-509 alone vs. ARN-509 + LHRH antagonist vs. LHRH antagonist alone	Mean change in QoL measured by total FACT-P score	Biochemical-relapsed hormone-sensitive prostate cancer	NCT01790126
	ARN-509	III	Randomized, placebo controlled	Metastasis-free survival	SPARTAN trial, nonmetastatic CRPC	NCT01946204
	ODM-201	I/II	Randomized, open label	Safety and tolerability	ARADES trial, dose-escalation study with a randomized phase II expansion component, mCRPC	[80, 81], NCT01317641 NCT01429064
	AZD3514	I	Randomized, open label	Safety and tolerability	mCRPC	NCT01162395
Hsp90	17-AAG	II	Open label	PSA response	Grade 3 toxicity included fatigue, lymphopenia, and back pain	[88]
	Ganetespib (STA-9090)	II	Single arm	PFS	Post-chemo mCRPC	NCT01270880
	AT13387	I/II	Randomized, open label, two arm: AT133887 + abiraterone vs. AT13387 alone	Phase A: safety, tolerability, optimal dosing; Phase B: response rate per PCWG2 [59] and CTC count between single agent and combo	Post-abiraterone mCRPC	NCT01685268

(continued)

Table 6.1 (continued)

Class	Agent	Phase	Treatment arms	Primary end point	Remarks and patient population	Reference/ClinicalTrials.gov identifier
Hsp27	OGX-427	II	Randomized, open label, two arm: OGX-427 + prednisone vs. prednisone alone	PFS (per PCWG2)	Chemo-naive mCRPC	NCT01120470
Antisense oligonucleotides	Custirsen (OGX-011)	III	Randomized, open label, two arm: docetaxel + prednisone + OGX-011 vs. docetaxel + prednisone	OS	SYNERGY trial, chemonaïve mCRPC Study completed. Did not meet primary endpoint of OS	NCT01188187 [112]
	Custirsen (OGX-011)	III	Randomized, open label, two arm: cabazitaxel + prednisone + OGX-011 vs cabazitaxel + prednisone	OS	AFFINITY trial, post 1st line chemo, mCRPC	NCT01578655
	EZN-4176	I	Non-randomized, open label	MTD and safety profile	AR mRNA antagonist, mCRPC	[111]
Epigenetic therapies	Azacitidine	I/II	Non-randomized, open label	MTD and safety profile	mCRPC previously treated with docetaxel	NCT00503984
Novel targets	TOK-001	II	Single arm open label	MTD, safety profile, and PSA response	ARMOR2 trial, mCRPC. Three mechanisms of action: inhibits CYP17, antagonizes testosterone binding to AR, degrades AR protein	NCT01709734

AR androgen receptor, *chemo* chemotherapy, *FACT-P* Functional Assessment of Cancer Therapy-Prostate, *Hsp* heat-shock protein, *LBD* ligand-binding domain, *LHRH* luteinizing-hormone-releasing hormone, *mCRPC* metastatic castration-resistant prostate cancer, *MTD* maximum tolerated dose, *OS* overall survival, *PCWG2* Prostate Cancer Working Group 2, *PFS* progression-free survival, *rPFS* radiographic progression free survival, *PSA* prostate-specific antigen, *QoL* quality of life, *RP2D* recommended phase 2 dosage

profile and failure to meet basic efficacy criteria, the trial was terminated and the development of BMS-641988 was discontinued [53].

Enzalutamide

Enzalutamide, formerly known as MDV3100, is a novel oral nonsteroidal AR antagonist developed to target oncogenic alterations associated with resistance to the first-generation antiandrogens. Compared to first-generation agents and BMS-641988, enzalutamide has a greater affinity towards the AR, displays no agonistic properties, and has a distinct ability to inhibit nuclear translocation, DNA binding, and coactivator recruitment (Fig. 6.1). In definitive phase III studies, enzalutamide was found to prolong survival in men with metastatic CRPC, improve their quality of life, and delay time to first skeletal-related event, leading to its approval by the United States Food and Drug Administration in 2012.

Preclinical Development

The development of enzalutamide began with the chemical scaffold molecule RU59063, a nonsteroidal AR agonist, due to its unique selectivity over other nuclear hormone receptors and its high affinity for the AR [54, 55]. Nearly 200 thiohydantoin derivatives of RU59063 were tested for AR agonism and antagonism in human prostate cancer cells engineered to express increased amounts of the AR. After further chemical modifications, including optimization of pharmacokinetic properties such as serum half-life and oral bioavailability, two compounds, diarylthiohydantoins RD162 and MDV3100, were chosen for further investigation [56, 57].

MDV3100 and RD162 both displayed a five- to eight-fold greater affinity for the AR compared with bicalutamide (measured in a competition assay using 16β-[^{18}F]-fluoro-5α-dihydrotestosterone [^{18}F-FDHT]) as well as an increased selectively to the AR (measured in an in vitro fluorescence polarization assay). Additional assays demonstrated that, when bound to the AR, both RD162 and MDV3100 impaired nuclear translocation, DNA binding, and coactivator peptide recruitment, while bicalutamide and DHT did not. Neither RD162 nor MDV3100 activated the wild-type AR or the mutant receptor that converts bicalutamide to a pure agonist (W741C); and in LNCaP/AR xenograft castrate mice, they both induced tumor regression while bicalutamide merely slowed tumor growth [57]. Further, cells treated with either RD162 or MDV3100 showed a five-fold reduction in the ratio of nuclear to cytoplasmic AR compared to bicalutamide-treated cells. Given its favorable drug-like properties and safety profile, MDV3100 was chosen over RD162 for further clinical development.

Phase I/II Trial

Given the encouraging preclinical data, a first-in-man, phase I/II dose-escalation trial was conducted to identify the safety and tolerability profile of MDV3100 as well as to establish the maximum tolerated dose (MTD) [58]. A total of 140 men with metastatic CRPC who were either chemotherapy naïve (n = 65) or had prior taxane exposure (n = 75) were enrolled. Antitumor effects were seen at all doses, including a ≥50 % PSA decline in 78 patients (56 %). Other antitumor effects were evaluated using the Prostate Cancer Clinical Trial Working Group 2 (PCWG2) criteria [59]. Time to biochemical progression, defined as an increase in PSA of ≥25 % from the nadir, was found to be 41 weeks for chemotherapy-naïve patients and 21 weeks for patients who had received prior taxane therapy. There was also a significant difference in radiographic progression between the two groups. For chemotherapy-naïve patients, median time to radiographic progress was not reached, whereas for taxane-pretreated patients it was 29 weeks.

Circulating tumor cells (CTCs) were also collected from 128 patients (91 %) before and after the administration of MDV3100. A cutoff of 5 cells per 7.5 ml of blood was used to determine favorable status (<5 cells) versus unfavorable (≥5 cells) [60]. Of 77 patients who had favorable CTC counts prior to treatment, 70

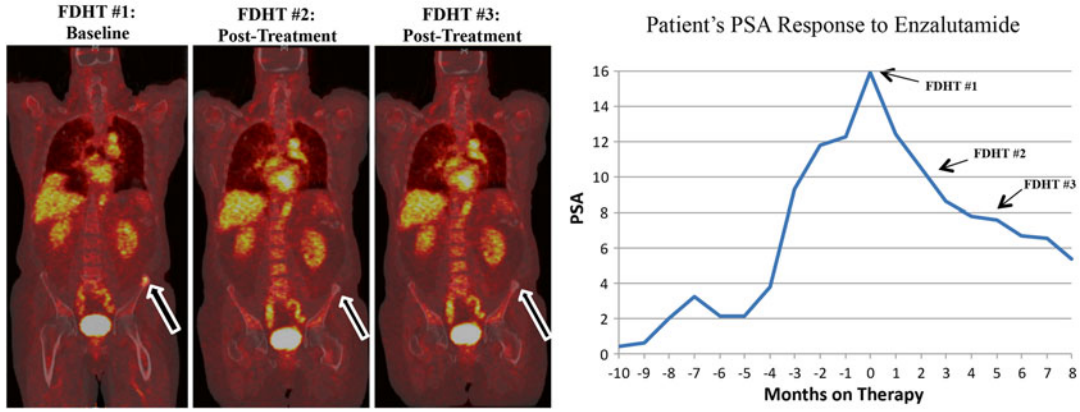

Fig. 6.2 A patient's response to enzalutamide on FDHT-PET and PSA. The *arrows* in the figure correlate to a left iliac bone lesion that was noted to have FDHT uptake prior to treatment with enzalutamide. On post-treatment analysis, the FDHT uptake as well as the patient's PSA declined revealing an on-target and treatment effect with enzalutamide

(91 %) maintained their counts during treatment, whereas 49 % who had unfavorable counts prior to treatment converted to favorable after treatment. CTC status has recently been shown to be an accurate predictor of survival, with a median OS of 21.7 months for patients with favorable pretreatment CTC count versus 11.5 months for patients with unfavorable CTC count [61, 62].

A subset of 22 of the 140 patients participated in an additional experimental biomarker trial, in which positron emission tomography (PET) imaging was used to measure uptake of ^{18}F-FDHT before and after treatment with MDV3100 [58], to assess AR blockade. An androgen analog, ^{18}F-FDHT localizes to tumor tissue and binds to the AR, allowing for direct visualization of the study drug and its target as well as potential identification of treatment-related variations in metastatic sites [63]. Figure 6.2 shows a scan of one patient enrolled on the phase I/II clinical trial evaluating MDV3100. The baseline FDHT-PET scan shows an overexpression of the AR on the left iliac crest through increased uptake of ^{18}F-FDHT. However, after treatment with MDV3100 the ^{18}F-FDHT uptake diminishes, as do the PSA levels (seen on the adjacent graph), revealing that the antiandrogen is inhibiting its target. All 22 patients showed a clear reduction in ^{18}F-FDHT uptake on post-treatment imaging. Interestingly, uptake of the radiotracer plateaued at a dose of 150 mg of MDV3100, even though higher plasma concentrations of the study drug were found at the higher doses, demonstrating that higher serum concentrations of MDV3100 do not correlate to increased levels of AR binding.

Toxic effects were graded with the National Cancer Institute Common Terminology Criteria for Adverse Events (NCI CTCAE version 3.0). Fatigue was the most common grade 3–4 adverse event, seen at doses ≥240 mg and occurring in 11 % of patients; it was also the most common grade 2 adverse event, seen in 27 % of patients. There were also three documented seizures seen at dosages from 360 to 600 mg. Even though antiandrogens as a class have been shown to cause seizures in animal models—through an off-target mechanism inhibiting GABA-A (gamma aminobutyric acid) channels—the patients who experienced seizure activity on trial had underlying comorbidities or were on medications that potentially could have contributed to the event [64]. Based on its tolerability profile, and activity as demonstrated by PSA, conventional imaging, and FDHT responses, a dose of 160 mg per day of MDV3100 was selected to be the optimal dose for further studies [58].

Phase III Trials

As a result of the positive phase I/II clinical trial of MDV3100, a multinational phase III double-blinded placebo-controlled trial (AFFIRM) was initiated to further evaluate the compound, now called enzalutamide, in 1,199 men with chemotherapy-treated CRPC. The trial accrued in 14 months with a 2:1 assignment, and was unblinded by an independent data safety monitoring committee after a planned interim analysis of 520 events was reached. At the time of analysis, a 37 % reduction in the risk of death was seen in the enzalutamide group compared with placebo (HR = 0.63; 95 % CI 0.53–0.75; $P < 0.001$) [65].

Overall, the AFFIRM trial showed an improvement in OS of 4.8 months (median OS 18.4 months in the enzalutamide group versus 13.6 months in the placebo group). In addition, an advantage of enzalutamide over placebo was seen in all secondary endpoints, including a reduction in PSA level by ≥50 % and improved soft-tissue response rate, time to PSA progression, quality-of-life response rate (using the Functional Assessment of Cancer Therapy-Prostate [FACT-P] questionnaire), radiographic PFS, and time to first skeletal-related event. The most common adverse effects were similar to the phase I/II trial and included fatigue (34 %), diarrhea (21 %), hot flashes (20 %), and seizures (<1 %). As a result, in 2012 enzalutamide was approved for clinical use in the USA in men with metastatic CRPC previously treated with docetaxel (Table 6.1).

More recently, the PREVAIL trial evaluated enzalutamide in 1,717 men with CRPC that progressed despite ADT but who had not yet received chemotherapy. A pre-planned interim analysis was conducted after 540 events (patient deaths) and found benefit in the treatment arm for which the study was terminated. Both primary endpoints, radiographic progression-free survival and overall survival, significantly favored the enzalutamide arm. The rate of radiographic progression-free survival was 65% in the enzalutamide arm versus 14% in the placebo arm (HR=0.19; 95%CI 0.15-0.23) at 12 months of treatment. In addition, 631/872 (72%) patients were alive in the enzalutamide arm compared to 546/845 (63%) patients in the placebo arm for a 29% reduction in the risk of death (HR=0.71; 95% CI 0.60-0.84). Enzalutamide was also found to be superior with respect to all the evaluated secondary endpoints. Fatigue remained the most common adverse effect seen in >33% of patients in the enzalutamide arm which was similar to the AFFIRM trial described above. Other common adverse effects were back pain (27%), constipation (22%) and arthralgia (20%). At the current data-cutoff date of January 15, 2014, 1 patient in both the enzalutamide and placebo arm experienced a seizure [66].

Mechanism of Resistance to Enzalutamide

Enzalutamide has been shown to improve OS, time to first skeletal-related event, and quality of life, but with a median time to radiographic progression of 8 months in the chemotherapy resistant population [65]. Currently, mechanisms of resistance to enzalutamide are under evaluation, although previously described mechanisms in CRPC, including AR overexpression, mutations in the AR, AR splice variants, and bypass pathways, are currently being investigated [10].

One area of potential resistance to enzalutamide is through upregulation of the glucocorticoid receptor, a nuclear receptor in the same family (NR3C) as the AR. In preclinical models it was found to be upregulated in enzalutamide-resistant mice, leading to the expression of a subset of targeted genes that drives prostate cancer growth. Administration of dexamethasone, a glucocorticoid receptor agonist, in an enzalutamide-resistant model suppressed glucocorticoid receptor function while the AR function was partially restored. Furthermore, suppression of the glucocorticoid receptor in mice treated with enzalutamide revealed further suppression in tumor growth compared to mice with normal glucocorticoid receptor function [67].

Another mechanism of resistance to enzalutamide has recently been described through a missense mutation in the LBD of the AR. The AR F876L mutation has been seen in cell lines, xenograft models, and plasma DNA of patients

treated with prolonged courses of enzalutamide. The F876L mutation is also associated with an enzalutamide antagonist-to-agonist switch similar to the mutation in codon 741 that is associated with the bicalutamide antagonist-to-agonist switch [47, 68–70]. Mutation F876L has also been shown to reveal resistance to ARN-509 (discussed below) [69, 70].

AR splice variants have also been hypothesized as a possible mechanism of resistance to enzalutamide through their ligand-independent AR transactivation [71]. Enzalutamide is able to antagonize androgen-mediated activation in cell lines that maintain a full-length AR, but the human prostate carcinoma 22Rv1 cell line, which contains an AR splice variant, is able to maintain its growth potential under castrate levels of androgen despite concurrent administration of antiandrogens such as bicalutamide and enzalutamide.

Finally, resistance to enzalutamide may be mediated through loss of *PTEN*, a tumor suppressor gene, or through activation of the PI3K/AKT/mTOR signaling pathway [72]. The two most frequently activated signaling pathways in prostate cancer are driven by PI3K and AR, and these pathways have been found to regulate each other through reciprocal feedback such that inhibition of either pathway alone causes activation of the other and protects tumor cells from death. The reciprocal feedback between the AR-directed pathway and the PI3K/AKT/mTOR pathway, however, can hypothetically be overcome by a combined treatment approach using an AR antagonist plus a PI3K/AKT/mTOR pathway inhibitor [73, 74]. This approach has been well studied in other malignancies, including breast cancer, with some treatment success [75], and multiple trials are currently studying this combined treatment approach for prostate cancer.

Future Areas of Interest for Enzalutamide

Now that enzalutamide has shown improvement in OS in metastatic CRPC independent of chemotherapy, future areas of research are opening. Enzalutamide is being studied in different prostate cancer populations including castration-sensitive disease. It is also being evaluated in combination with other therapeutic agents, including abiraterone acetate (a CYP17 inhibitor), tivozanib (a VEGF receptor tyrosine kinase inhibitor), and sipuleucel-T (cellular immunotherapy). A phase III randomized clinical trial comparing enzalutamide to enzalutamide plus abiraterone acetate and prednisone in the chemotherapy-naïve population has recently opened as a collaborative study led by the Alliance for Clinical Trials in Oncology (ClinicalTrials.gov Identifier: NCT01949337).

ARN-509

Even as the development of enzalutamide continues to progress into new clinical arenas, newer AR antagonists have shown promising results. ARN-509 is a next-generation antiandrogen with full antagonistic potential, high affinity to the AR (seven- to ten-fold greater affinity compared to bicalutamide), and the ability to limit AR nuclear translocation and AR binding to androgen response elements. When compared to enzalutamide in preclinical models, ARN-509 achieved maximum efficacy at a lower dose (10–30 mg/kg/d for ARN-509 compared to 30–100 mg/kg/d for enzalutamide). In addition, preclinical models showed an approximately two- to four-fold lower steady-state plasma concentration for ARN-509 compared to the equivalent dose of enzalutamide; at the same time, intratumoral levels of ARN-509 and enzalutamide were roughly equivalent, indicating a higher tumor/plasma ratio with ARN-509. Furthermore, after 28 days of therapy with either ARN-509 or enzalutamide, steady-state brain tissue levels were measured in mice and there was a fourfold lower level in the ARN-509 group compared to the enzalutamide group, possibly indicating a lower seizurogenic potential [76].

Results of the phase I portion of an ongoing phase I/II clinical trial of ARN-509 in men with CRPC have recently been published, and the phase II portion has recently completed accrual (ClinicalTrials.gov Identifier: NCT01171898) [77]. The trial, which evaluated safety, MTD, and antitumor activity at doses ranging from 30 to 480 mg a day, found that ARN-509 is safe and

well tolerated with the most common side effect being grade 1–2 fatigue, which was seen in 14/30 patients (47 %). One grade 3 dose-limiting toxicity event, abdominal pain, was seen at the 300 mg/day dose. Through the use of FDHT-PET, AR binding was seen at all doses, with a plateau response at doses of ≥120 mg a day. However, the steady-state concentration of ARN-509 at the 120 mg daily dose was less than the concentration needed for tumor regression in the preclinical model, so 240 mg a day was chosen as the dose for the phase II clinical trial. Of the patients treated on the phase I clinical trial, 47 % had a ≥50 % decline in their PSA by 12 weeks, indicating possible efficacy.

Preliminary analysis of the phase II trial has found a ≥50 % decline in PSA at 12 weeks in 91 % of patients with nonmetastatic CRPC who are treatment naïve (no prior chemotherapy or second-generation antiandrogen), 88 % who have treatment-naïve metastatic CRPC, and 29 % who have post-abiraterone, chemotherapy-naïve metastatic CRPC. Although analysis is still ongoing, preliminary data is encouraging, demonstrating activity in both nonmetastatic and metastatic chemotherapy-naïve CRPC, both before and after abiraterone acetate [78, 79]. As a result, the SPARTAN trial, a phase III randomized double-blinded placebo-controlled trial in men with nonmetastatic CRPC, is now open and accruing (ClinicalTrials.gov Identifier: NCT01946204).

Future Approaches

Understanding why some prostate cancers do not respond to the newer antiandrogens or why some do not maintain a durable response will be key to guiding further developments within the field. There are a number of clinical trials currently testing novel agents that enhance AR-ligand pathway inhibition, disrupt AR activation, and block downstream transcription that are described below.

Novel AR Inhibitors

With a mechanism of action similar to enzalutamide and ARN-509, ODM-201 displays a relatively high affinity for the AR and lacks the partial agonist activity seen in bicalutamide. However, unlike the newer antiandrogens, ODM-201 has a negligible tissue/plasma ratio in mouse brain models, thus theoretically eliminating the seizure potential that is a side effect of this class of agents. Data from the phase I component of the ARADES study, a first-in-man open-label phase I/II clinical trial evaluating ODM-201 in patients with progressive metastatic CRPC, indicated no dose-limiting toxicity. A ≥50 % decline in PSA was obtained in 13/15 patients (87 %) who were evaluated at 12 weeks. In addition, 92 % of the abiraterone-naïve/chemotherapy-naïve patients had a ≥50 % decline in PSA, versus 86 % of abiraterone-naïve but chemotherapy-exposed patients. The most frequent grade 1–2 adverse events were asthenia, diarrhea, and nausea. The phase II, dose-escalation expansion component of ARADES was recently presented at the European Cancer Congress 2013 in Amsterdam. There was a ≥50 % decline in PSA in 65 % of chemotherapy- and abiraterone-naïve patients, 32 % of post chemotherapy and abiraterone-naïve patients, and 9 % of post-abiraterone patients. The most common grade 1–2 side effects were fatigue (24 %), back pain (14 %), and constipation (13 %). No seizure activity was seen during study treatment (ClinicalTrials.gov Identifier: NCT01317641 and NCT01429064) [80, 81].

Targeting the AR Protein Chaperones

Unlike enzalutamide and ARN-509, which target the AR, some new agents target the AR signaling axis through direct disruption of the AR protein chaperones. Hsp are stress proteins that function as molecular chaperones to regulate protein homeostasis. They play a role in different signaling pathways and transcriptional survival networks that help facilitate cell survival and inhibit apoptosis. These molecular chaperones, particularly Hsp90, assist in the specific folding, trafficking, activation, and transcriptional activity of the AR that leads to tumor cell survival in CRPC [82, 83].

Hsp90, along with other co-chaperones, maintains the AR in a high affinity ligand-binding conformation that facilitates efficient response to DHT. 17-N-allylamino-17-demethoxygeldanamycin (17-AAG) is a small molecule inhibitor of the Hsp90 chaperone protein. Preclinical data showed that 17-AAG resulted in the inhibition of prostate cancer cell proliferation, downregulation of the AR, reduc-

tion of AR expression in prostate cancer xenograft tumors, and suppression of growth in so-called androgen-dependent and androgen-independent prostate cancers [84]. Phase I trials using 17-AAG were initiated with different dosing regimens and in combination with chemotherapy. DLTs included hepatotoxicity, cytopenia, fatigue, myalgias, and nausea, and intermittent dosing schedules were found to be overall less toxic [85–87]. However, when expanded into a phase II trial in metastatic CRPC, 17-AAG did not show sufficient PSA response or objective disease response as evaluated by radiologic imaging studies, and enrollment was terminated [88]. Other Hsp90 inhibitors, including IPI-504, have either failed to show antitumor activity or resulted in significant toxicity in prostate cancer clinical trials [89]. However, more recently, modifications in the target, such as inhibition of Hsp90 cofactors like FKBP52, as well as agents with better tolerated toxicity, are currently being evaluated in phase I/II trials [90, 91].

OGX-427 is a second-generation 2′-methoxyethyl modified phosphorothioate antisense oligonucleotide that targets Hsp27, also called HspB1. A potent antiapoptotic molecule highly expressed in CRPC, Hsp27 regulates AR stability, nuclear translocation, and transactivation [92]. A phase II trial investigating OGX-427 with or without prednisone in 72 patients with chemotherapy-naïve metastatic CRPC (ClinicalTrials.gov Identifier: NCT01120470) found at 12 weeks that 71 % of patients receiving OGX-427 plus prednisone were progression free, compared to 40 % receiving prednisone alone. In addition, 50 % of patients in the OGX-427 plus prednisone arm had a ≥50 % decline in PSA, compared to 20 % in the prednisone-only arm. The study, which was presented at the 37th European Society for Medical Oncology Congress, also showed positive benefit when evaluating for measurable disease response and CTC conversion [93].

Custirsen (OGX-011), another second-generation 2′-methoxyethyl modified phosphorothioate antisense oligonucleotide, has been found to suppress clusterin, a cytoprotective chaperone protein with heat shock-like properties, in preclinical models. Clusterin is upregulated in stress states such as androgen deprivation and chemotherapy and is thought to play a role in chemotherapy-refractory disease [94–98]. Two phase II trials evaluating OGX-011 in metastatic CRPC have shown positive results, and as a result, OGX-011 is currently being evaluated in a phase III clinical trial in combination with second-line chemotherapy (Table 6.1) [99, 100].

Agents with Novel Targets

Galeterone (TOK-001), an orally available semisynthetic steroid analog, has a unique mechanism of action against inhibiting prostate cancer cell growth. The novel agent works through a triple mechanism of action, by inhibiting the human CYP17A1 enzyme, behaving as an AR antagonist to both the wild-type and mutant AR, and degrading the AR protein [101–103]. A phase I trial, ARMOR1, evaluated galeterone in 49 men with either metastatic or nonmetastatic chemotherapy-naïve CRPC that had progressed through ADT. Patients were randomized to receive either single or split doses of galeterone, ranging from 650 to 2,600 mg daily for 12 weeks [104]. The MTD was not reached. The most common grade 1–2 adverse events were fatigue (37 %), liver function test abnormalities (31–33 %), nausea (29 %), diarrhea (27 %), and pruritus (25 %). One related grade 4 toxicity occurred, involving rhabdomyolysis in the setting of concurrent statin use and underlying renal insufficiency. PSA reduction of ≥30 % was seen in 24 patients (49 %), including 11 (22 %) who had a ≥50 % reduction in PSA. ARMOR2, a phase II clinical trial, has opened for accrual (Table 6.1) (ClinicalTrials.gov Identifier: NCT01709734).

Epigenetic Therapies

Yet another approach to treatment of CRPC is through targeting the transcriptional function of the AR. As previously stated, the AR is a transcription factor that plays an important role in the normal development and maintenance of the prostate gland as well as in tumorigenesis. Preclinical studies have shown promising results targeting epigenetic alternations using histone deacetylase inhibitors (HDACi) and DNA methyltransferase (DNMT) inhibitors.

HDACi have been found to be effective in inhibiting tumor proliferation and/or inducing cellular apoptosis in preclinical models. However, despite promising preclinical results using cell lines and in vivo models, initial clinical trials have not demonstrated significant efficacy. Vorinostat, an oral small molecule inhibitor of class I and II HDACs, was evaluated in a phase II clinical trial of 27 patients with metastatic CRPC pretreated with chemotherapy. The best radiologic response was stable disease in two patients (7 %). There was no significant PSA response, and 44 % of patients experienced grade 3 adverse events, the most common being fatigue (81 %), nausea (74 %), and anorexia (59 %) [105]. Panobinostat, a pan-deacetylase inhibitor, was studied in intravenous form in a phase II clinical trial in docetaxel-refractory metastatic CRPC. Of the 35 patients enrolled, none achieved a PSA decline ≥50 %, and panobinostat was associated with fatigue (62.9 %), thrombocytopenia (45.7 %), nausea (51.4 %), and decreased appetite (37.1 %) [106]. Currently available HDACi are limited by their inability to achieve sufficient drug concentrations in vivo without excessive toxicity, and because the majority are nonselective, leading to off-target effects. Thus, laboratory research is focused on establishing more-sensitive HDACi—such as tubacin [107] and SB-429201 [108], which target only the HDAC6 and HDAC1, respectively—as well as combining HDACi with other currently available therapeutic modalities, including radiotherapy, chemotherapy, AR antagonists, and apoptosis- and angiogenesis-inducing agents.

DNMT inhibitors are also under evaluation for the treatment of patients with mCRPC. Azacitidine, a subcutaneously administered hypomethylating agent, was found in preclinical experiments to reverse resistance in hormone- and chemotherapy-refractory models [109]. A phase II clinical trial evaluating azacitidine in 34 patients with chemotherapy-naïve metastatic CRPC showed a significant prolongation of PSA doubling time compared to baseline, as well as a median PFS of 12.4 weeks. Side effects of azacitidine included fatigue (41.2 %) and neutropenia (17.6 %) [110]. Currently a phase I/II clinical trial of azacitidine in combination with docetaxel and prednisone is evaluating MTD as well as PSA and radiologic response in patients with chemotherapy-refractory metastatic CRPC (ClinicalTrials.gov Identifier: NCT00503984).

Conclusion

Targeting the AR in CRPC has led to the discovery and subsequent approval of enzalutamide as treatment for a population at high risk of morbidity and mortality. However, despite the recent advances in AR-targeted therapy, it is of utmost importance that we continue to identify areas of resistance and new treatment targets. We must continue to develop our understanding of the AR-ligand pathway and, in parallel, develop biomarkers to prognosticate, predict and monitor response to novel AR-targeted agents, which will improve clinical outcomes, palliate symptoms, and extend life.

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Approaches Targeting Androgen Synthesis (CYP 17 Inhibitors)

7

Adam Siegel and Charles J. Ryan

Introduction

Prostate cancer growth is highly dependent on androgen signaling; consequently, androgen deprivation therapy (ADT) is the initial recommended treatment approach for patients with metastatic prostate cancer. Historically, ADT was achieved with surgical or pharmacologic castration with diethylstilbestrol. These interventions have largely been supplanted by luteinizing hormone-releasing hormone (LHRH) therapy. Approximately 90 % of men experience an initial response to ADT as measured by normalization of prostate specific antigen (PSA) and objective tumor responses.

Response to ADT is largely attributed to suppression of hypothalamic–pituitary–gonadal axis testosterone production. Ultimately, continued ADT results in emergence of tumor that is more sensitive to lower levels of androgen and is capable of continued androgen-dependent growth despite castrate testosterone levels (i.e., typically defined as testosterone less than 50 ng/dL). This confluence of tumor progression despite castrate levels of androgen has driven the emergence of the definition of this clinical state as castrate-resistant prostate cancer (CRPC).

Androgen Production and Continued AR-Dependent Signaling in the Castrate-Resistant State

Although LHRH agonists and antagonists suppress circulating serum testosterone levels by 85–90 %, adrenal and intratumoral androgenesis is relatively unaffected. Androstenedione and dihydroepiandrostenedione (DHEA) are the predominant adrenal androgen derivatives. These may ultimately serve as weak AR ligands, or ultimately as substrates for conversion to testosterone and dihydrotestosterone (DHT).

Androgen receptor (AR)-dependent cell signaling has been increasingly implicated in the pathogenesis of metastatic castrate sensitive prostate cancer (mCRPC). Multiple mechanisms have been identified by which androgen-dependent signaling pathways remain active, including AR amplification, AR mutation with subsequent sensitization to weaker endogenous ligands, upregulation of transcriptional co-activator proteins, or AR signal augmentation induced by other receptor pathways [1]. Continued AR stimulation underlies each of these mechanisms, typically by low levels of circulating

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F. Saad and M.A. Eisenberger (eds.), *Management of Castration Resistant Prostate Cancer*, Current Clinical Urology, DOI 10.1007/978-1-4939-1176-9_7,

adrenal androgens (endocrine source) or intracellular androgens (intracrine source).

Increased intracellular androgen synthesis has been proposed as one mechanism by which castrate-resistant prostate cancer cells maintain adequate intracellular androgen for continued AR-dependent signaling. Despite the significant ADT-induced reduction in circulating serum androgens, the concomitant reductions in intraprostatic testosterone and dihydrotestosterone levels are lower. Although androgen deprivation in otherwise healthy men results in an approximately 90 % reduction in circulating serum androgens, this reduction coincides with only a 70–80 % reduction in intratumoral androgens, with adequate residual intracellular androgen levels for continued AR-dependent signaling [2]. Recent work has revealed higher levels of androgens and transcripts encoding androgen-synthesizing enzymes within mCRPC metastatic lesions versus metastatic lesions from men with untreated prostate cancer [3]. This problem is not limited merely to de novo, intracellular androgen synthesis: The increased expression of AKR1C3 and SRD5A1, genes encoding prostatic enzymes capable of converting adrenally produced androstenedione to testosterone and DHT, has similarly been implicated as one mechanism by which mCRPC tissue sustains adequate ligand for the AR [4, 5]. In effect, the tumor can become a "sink" for concentration of androgens as well as a source of intracrine androgen production and conversion to active forms. These data support the notion that novel AR directed therapies not only target the endocrine sources but also target the tumor directly in these highly adapted tumors.

CYP 17A1 as a Clinical Target in CRPC

CYP 17A1 is a multifunctional cytochrome p450 enzyme localized within the endoplasmic reticulum of the adrenal glands and testes, where it serves to catalyze multiple steps in androgen biosynthesis. The enzyme first facilitates conversion of pregnenolone and progesterone to 17-hydroxypro gesterone and 17-hydroxypregnenolone (17-αhydroxylase activity), with subsequent catalysis of these precursors to DHEA and androstenedione (C-17,20 lyase activity), respectively. While these functions are also involved in glucocorticoid biosynthesis, CYP 17A1 inhibition does not typically result in overt glucocorticoid deficiency: Inherent adrenal feedback induces increased ACTH secretion and downstream production of weak glucocorticoids including corticosterone (see Fig. 7.1). This phenomenon is best demonstrated in individuals with congenital CYP 17 deficiency, which typically presents with ambiguous genitalia without clinically significant glucocorticoid deficiency.

The successful treatment of breast cancer with the CYP 19 inhibitor exemestane (Aromasin) provided a promising precedent for the treatment of other hormone-sensitive cancers [6]. CYP 17A1 plays an analogous role in prostate cancer growth, as it serves to catalyze androgen biosynthesis in the adrenal glands and testes. In light of the increasing evidence supporting continued androgen-dependent cell signaling even in the castrate-resistant state, CYP 17A1 inhibition serves as an attractive therapeutic target.

Ketoconazole

Ketoconazole, an imidazole-derived anti-fungal medication, was first noted in the 1980s to induce gynecomastia in patients treated for non-prostate cancer indications. High dose ketoconazole (HDK) therapy was later demonstrated to significantly reduce testosterone, androstenedione, and DHEA levels in treated patients [7]. Ketoconazole is a weak inhibitor of multiple steps in androgen biosynthesis including CYP 17A1 mediated 17-αhydroxylase and C-17,20 lyase activity, as well as 11β-Hydroxylase-dependent corticosterone and cortisol synthesis, 14αdemethylase catalysis of lanosterol conversion to cholesterol, and desmolase-mediated conversion of cholesterol to pregnenolone. Thus, HDK therapy is commonly associated with clinical glucocorticoid and mineralocorticoid deficiency, necessitating concomitant administration of exogenous glucocorticoids. Despite the significant risk of

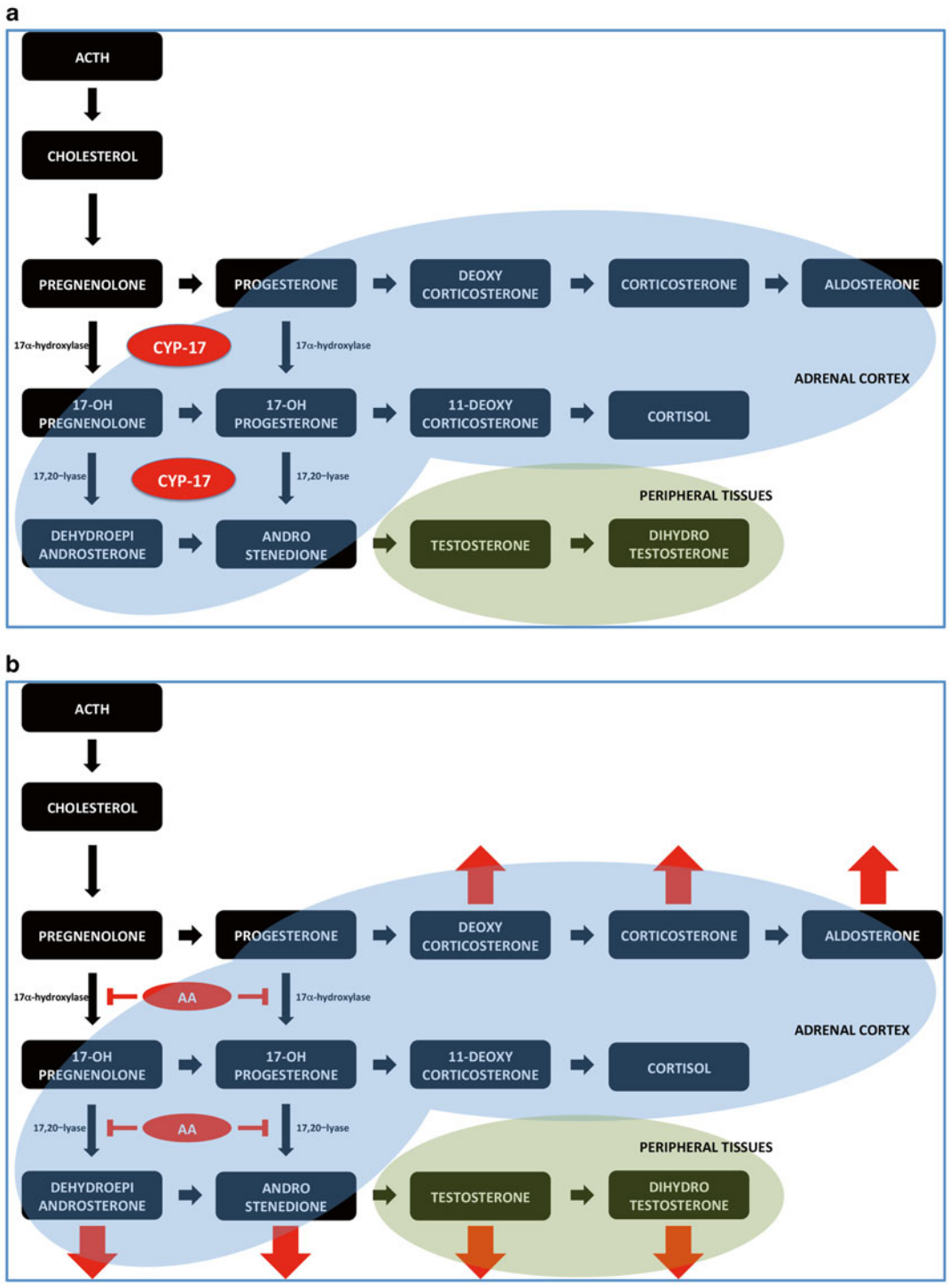

Fig. 7.1 Schema showing mechanism of action of CYP 17 and CYP 17 Inhibitor abiraterone acetate (AA). Note that upon inhibition of CYP 17 the mineralocorticoid path can be activated, thus leading to Na+ retention and K+ loss. Coadministration with prednisone reduces these effects

side effects, ketoconazole is widely available as an inexpensive generic, and has been widely used off-label in the treatment of mCRPC in the USA.

Multiple clinical trials evaluated the efficacy and toxicity of HDK in patients with CRPC. Initial phase 1 and 2 trials implementing HDK therapy demonstrated that 40–62.5 % of patients exhibited an initial PSA decline of greater than 50 %. Nonetheless, duration of response to HDK therapy in these trials was brief, with median duration of response (i.e., as defined by PSA rise or radiographic evidence of progression) ranging from only 3.3 to 8.5 months [8–10].

These initial trials were later followed by CALGB-9583, a randomized phase III trial of anti-androgen withdrawal (AAWD) alone or in combination with ketoconazole 400 mg administered three times daily and hydrocortisone 30 mg orally every morning and 10 mg each evening [11]. PSA response rates were 11 % in patients treated with AAWD alone and 27 % in patients treated with AAWD with ketoconazole. There was no significant difference in overall survival. However, 82 % of patients in the AAWD-alone treatment arm did ultimately receive deferred ketoconazole therapy, which may have mitigated any observable overall survival benefit. Within the AAWD arm, patients demonstrating a prior PSA response to AAWD (i.e., greater than 50 % decline) demonstrated a higher likelihood of subsequent PSA response to HDK as compared to those patients without a PSA response to AAWD (i.e., 67 versus 21 %, respectively). Patients in both arms of the study experienced toxicities including fatigue and malaise, neurologic toxicity, and gastrointestinal side effects, with a significantly greater proportion of patients in the ketoconazole arm experiencing grade 3 or 4 toxicity (21 versus 7 %). Despite the lack of an observable overall survival benefit, patients within the AAWD arm plus ketoconazole did demonstrate a relatively longer time to PSA progression (8.5 versus 5.9 months).

Serum testosterone and adrenal androgen levels (DHEA, DHEAS, and androstenedione) were similarly assessed at baseline, after 1 and 3 months of therapy, and at the time of progression. Patients in the AAWD and ketoconazole group demonstrated a significant decline in all 3 adrenal androgen levels; however, the serum testosterone levels did not change from baseline in either treatment arm. Subsequently, levels of all 3 adrenal androgens rose at the time of PSA progression, suggesting probable tachyphylaxis to ketoconazole [11]. A follow-up study later identified that elevation of baseline androstenedione levels were predictive of PSA response and overall survival in patients treated with ketoconazole [12], suggesting the need for further assessment of the predictive and prognostic value of serum androgens in the castrate-resistant clinical state.

The ongoing pathophysiologic importance of circulating adrenal androgens was further suggested by a concurrent study within which low-level accumulation of sensitizing AR ligand-binding site point mutations was identified in tumor tissue from patients demonstrating progression after AAWD. While there was neither association between detection of these point mutations and overall survival nor likelihood of progression, these findings did suggest androgen receptor sensitization as one mechanism leading to disease progression [13].

Despite its significant adverse effects and short duration of efficacy, ketoconazole remains an option in clinical use. Its advantages include availability as an inexpensive and generic medication, and its toxicities can be effectively managed with exogenous glucocorticoid administration. While the efficacy data from CALGB-9583 supported its continued clinical use, the breadth of its toxicities urged the subsequent development of highly specific CYP 17A1 inhibitors.

Abiraterone

Abiraterone acetate is an orally bioavailable, highly selective CYP 17A1 inhibitor with current FDA approval for treatment of both chemotherapy-naïve and chemotherapy-refractory mCRPC. It was initially developed through the Institute of Cancer research (ICR) at the Royal Marsden Hospital, where researchers implemented computational modeling to synthesize candidate steroid-derivative CYP 17A1 inhibitors.

Focus turned to a progesterone derivative, CB7598—later termed abiraterone—with preclinical in vivo testing in abiraterone-treated mice models demonstrating significant weight reductions in androgen-responsive organs including the prostate, seminal vesicles, and testes [14].

Phase I/II Clinical Trials

A series of small, phase I clinical trials were later published, with administration of single-dose abiraterone therapy to castrate men with prostate cancer in one trial, and administration of single-dose and dose-escalated abiraterone to non-castrate men with prostate cancer in an additional two trials [15]. In patients with castrate testosterone levels, a single 500 mg dose of abiraterone was adequate to significantly suppress serum testosterone levels. Nonetheless, non-castrate males receiving a single dose of abiraterone later demonstrated an endogenous luteinizing hormone (LH) surge, with a subsequent *rise* in serum testosterone levels. These findings have suggested the need for coadministered LHRH agonists in combination with abiraterone in subsequent clinical testing.

In contrast to single-dose abiraterone, daily dosing of 800 mg given on days 1–12 to non-castrate males was sufficient to achieve durable testosterone suppression. Of note, abiraterone administration was not associated with a significant decrease in serum cortisol levels; however, patients treated with abiraterone did demonstrate an abnormal ACTH stimulation test by day 11, suggesting subclinical disruption of adrenal glucocorticoid biosynthesis. While this series of phase 1 trials provided valuable insight into the pharmacokinetics of abiraterone, it did not explore clinical outcomes such as treatment response nor survival.

Subsequent phase I clinical testing evaluated the toxicity and safety of abiraterone, with a goal of establishing a safe and effective dose for subsequent phase II clinical studies. This was an open label, dose-escalation study with preplanned dosing of once daily abiraterone in doses ranging from 250 to 2,000 mg. The mineralocorticoid antagonist eplerenone was administered for toxicity secondary to treatment-associated mineralocorticoid excess; namely, hypertension, hypokalemia, and fluid overload. Dexamethasone administration was added for refractory symptoms secondary to mineralocorticoid excess. Greater than 50 % declines in serum PSA levels were observed after one month of therapy in over half of patients, and no treatment-related grade 3 or 4 toxicities were observed. Further, circulating serum androgens including DHEA, DHEA-S, androstenedione, and testosterone were significantly decreased after one month of therapy, with persistence of androgen suppression even at the time of PSA or radiologic progression [15].

A concurrent phase I dose-escalation study of abiraterone was conducted in the USA in which 33 men with chemotherapy-naïve CRPC with or without a prior history of ketoconazole therapy received abiraterone acetate in doses ranging from 250 to 1,000 mg daily. Again, exogenous glucocorticoid coadministration was reserved for patients with symptoms of adrenal insufficiency and fatigue, with administration of aldosterone antagonists only for those patients with symptomatic mineralocorticoid deficiency. Significant PSA responses were observed, with over half of patients experiencing a PSA decline of greater than 50 %. No dose limiting toxicities were observed. Of note, in those patients with a history of disease progression while on ketoconazole, 47 % demonstrated PSA decreases of ≥50 % [16] when treated with abiraterone. This finding provided in vivo evidence of the significantly higher potency of abiraterone versus ketoconazole, as suggested by prior in vitro evaluation in human testicular microsomes.

These phase I studies demonstrated a broad spectrum of clinical activity in chemotherapy-naïve patients with mCRPC, including those individuals with a history of progression on ketoconazole therapy. This supported further evaluation with phase 2 clinical trials, including evaluation of efficacy in both chemotherapy-naïve and chemotherapy-refractory patients.

The efficacy of abiraterone acetate in chemotherapy and ketoconazole-naïve patients was subsequently evaluated in a phase II multicenter

study, within which all patients received abiraterone acetate 1,000 mg daily with prednisone 5 mg twice daily [19]. A ≥50 % PSA decline after 3 months of therapy was observed in 22 of the 33 (67 %) patients and the median duration of treatment exceeded 1 year. Treatment was generally well tolerated, as no dose limiting toxicities were reported and severe toxicities were rare: Grade 3 fatigue, fluid retention, dizziness, hypokalemia, and hypertension were each reported in only one patient.

Abiraterone was further evaluated in concurrent phase II trials assessing its safety and efficacy in patients with progressive, chemotherapy-refractory mCRPC. Two multicenter, phase II studies were conducted evaluating patients with progressive mCRPC with disease progression after docetaxel-based chemotherapy. Patients were treated with either abiraterone acetate 1,000 mg daily with prednisone coadministration reserved for those patients with clinical glucocorticoid deficiency or mineralocorticoid excess [18], or with abiraterone acetate 1,000 mg daily with prednisone 5 mg twice daily, regardless of side effects status [17]. PSA declines of greater than 50 % were observed in 51 and 36 % of patients, and partial radiographic responses were observed in 27 and 18 % of those patients with radiographically evaluable disease, respectively [17, 18]. These trials were the first to reliably demonstrate efficacy of a hormonally directed agent in the chemotherapy-refractory mCRPC state.

Dose limiting toxicities were not observed in either study [17, 18]; however, the coadministration of prednisone resulted in a particularly low occurrence of grade 3 and 4 toxicity, with grade 3 fatigue observed in only 1 of the 58 (2 %) patients [17]. Anorexia, hypokalemia, and fatigue were slightly more common when abiraterone was administered without prednisone [18]. These findings, along with the phase II toxicity data from coadministration of low-dose prednisone with abiraterone in chemotherapy-naïve mCRPC patients, ultimately supported exogenous glucocorticoid coadministration for all patients as the standard for future clinical trials. Ongoing studies are evaluating whether 5 mg daily of prednisone is sufficient versus the standard 10 mg daily dose.

Pre- and post-treatment circulating tumor cells (CTC) were enumerated in both clinical trials in the chemotherapy-refractory mCRPC state [17, 18]. Of the 42 patients [17] and 27 patients [18] with unfavorable pre-treatment CTC counts (i.e., ≥5/7.5 mL) [20], 11 (41 %) and 10 (34 %) converted to favorable CTC counts (i.e., <5) following treatment [17, 18, 20]. These data reiterated findings in previous studies and supported future evaluation of CTC enumeration as a candidate biomarker in mCRPC.

Phase III Clinical Trials

The encouraging efficacy data from these phase II studies urged the need for phase 3 trials, both in the chemotherapy-naïve and chemotherapy-refractory state.

Given general agreement that testing abiraterone in the chemotherapy-refractory setting was the shortest route to regulatory approval and promised to benefit those patients in the greatest clinical need, the first phase III study to launch was in this population, COU-AA-301, was a randomized, placebo-controlled, multicenter phase 3 clinical trial which established the efficacy of abiraterone acetate in the chemotherapy-refractory mCRPC clinical state [21]. A total of 1,195 patients with progressive mCRPC after having received docetaxel-based chemotherapy were randomized in a 2:1 ratio to receive either abiraterone acetate 1,000 mg daily with prednisone 5 mg twice daily or placebo with prednisone 5 mg twice daily. An overall survival (OS) benefit was observed for those patients receiving abiraterone acetate with prednisone, with a median OS of 14.8 versus 10.9 months. Multivariate analysis confirmed the OS benefit (hazard ratio for death, 0.66) [21]. All secondary endpoints met significance, including treatment-associated PSA decrease of greater than or equal to 50 % from baseline, time to PSA progression, and radiographic evidence of progression free survival in patients with RECIST-evaluable soft tissue metastatic disease [21]. These results supported

widespread regulatory approval for abiraterone acetate in the chemotherapy-refractory, mCRPC clinical state.

COU-AA-301 was also the first to assess abiraterone-associated symptom palliation. Exploratory endpoints included assessment of intensity of pain and functional limitations secondary to pain via the Brief-Pain Inventory-Short Form (BPI-SF) [21], and were later reported as a secondary analysis [22]. BPI-SF assessments occurred on day 1 of each 28-day treatment cycle, with a median length of follow-up of 20.2 months. Slightly less than half of the patients enrolled reported significant disease related pain at baseline. Patients treated with abiraterone with prednisone experienced more rapid palliation of symptoms than patients treated with placebo with prednisone (5.6 versus 13.7 months) and a higher overall response rate in pain palliation (45 versus 28.8 % response) [22]. Further, time until first skeletal-related event (i.e., defined as occurrence of a pathologic fracture, spinal cord compression, or requirement for palliative radiation or surgical intervention for bony metastatic disease) was significantly longer for patients treated with abiraterone with prednisone versus placebo with prednisone (25.0 versus 20.3 months) [22].

Secondary analyses of the data from the 301 study continue to be performed and published and will inform the clinical use of this agent as well as the further development of several new agents in mCRPC.

One analysis was performed assessing the prognostic and predictive value of baseline serum androgen levels prior to abiraterone therapy. Because its principal mechanism of action is androgen reduction, it has been hypothesized that measurable androgen may be associated with outcome in CRPC patients. Using the 301 data set, assessment of baseline serum androgen levels (DHEA-S, testosterone, androstenedione) was performed via ultrasensitive liquid chromatography. Interestingly, regardless of the treatment administered, overall survival was greater in patients with higher baseline androgen levels [23]. In a subsequent secondary analysis of COU-AA-301, androgen detection by ultrasensitive liquid chromatography revealed a decrease in serum androgens by approximately 90 % in patients treated with abiraterone and prednisone, versus an approximate 50 % median decline in patients treated with prednisone alone [24]. These findings correlate with our mechanistic understanding of abiraterone's role in suppression of androgen biosynthesis, and mCRPC as a hormonally mediated state. Furthermore, this perhaps reflects evolution of a more aggressive biologic phenotype in an environment of low serum hormonal input, with potential implications for serum androgens as a possible prognostic and/or predictive biomarker in future mCRPC trials. Further study of the relationship of ligand to treatment efficacy and prognosis is ongoing.

Whereas COU-AA 301 demonstrated the efficacy of abiraterone in patients with chemotherapy-refractory disease, COU-AA 302 demonstrated the efficacy of abiraterone in the pre-chemotherapy mCRPC clinical state. This double-blind RCT randomized chemotherapy-naïve mCRPC patients in a 1:1 ratio to receive either abiraterone acetate 1 g daily in combination with prednisone 5 mg twice daily, or placebo with prednisone 5 mg twice daily. Predetermined efficacy endpoints included OS and radiographic progression free survival (rPFS) as assessed by CT scan, MRI, or bone scan. The study was unblinded after a planned interim analysis demonstrated a significant prolongation of rPFS in patients receiving abiraterone (16.5 versus 8.3 months), with an associated trend toward overall survival benefit [25]. In the subsequent interim analysis, the median survival of the abiraterone arm was determined to be 35.3 months compared to 30.1 months in the placebo arm, —this was associated with a Hazard Ratio of 0.79 (range 0.66–0.96) and a p value of 0.0151. The OS data did not cross the pre-specified criterion for statistical significance, a finding that may have been the result of confounding by a high rate of subsequent therapy in the placebo arm (including abiraterone). The study is regarded as positive in the aggregate based on this strong trend and the cumulative data on rPFS. As a result, the use of abiraterone acetate therapy in the pre-chemotherapy mCRPC clinical state received regulatory approval in the USA in late 2012.

Toxicity assessment in COU-AA-302 revealed relatively more frequent occurrence of fatigue, arthralgia, hepatotoxicity, and peripheral edema in the abiraterone versus placebo treatment arms [25]. Further, grade 1 and 2 mineralocorticoid associated toxicities were more common with abiraterone, including hypertension (22 versus 13 %), hypokalemia (17 versus 13 %), and fluid retention/edema (28 versus 24 %). Nonetheless, there was no significant difference in grade 3 or 4 mineralocorticoid associated side effects [25].

Quality-of-life and patient reported outcomes from the trial have been reported. In this series of analyses, the asymptomatic (BPI-SF score of 0 or 1) or minimally symptomatic patients (BPI-SF score of 2 or 3) were assessed for time to pain progression via sequential BPI-SF assessment. Health-related quality of life was concurrently assessed with the Functional Assessment of Cancer Therapy-Prostate (FACT-P) questionnaire at baseline and day 1 of each 28-day treatment cycle [26]. Results from this analysis revealed a significant delay in time to progression of mean pain scores (26.7 versus 18.4 months) and a trend towards benefit for delay to time until progression of worst pain scores (26.7 versus 19.4 months). Further, patients in the abiraterone treatment arm reported a significantly longer time to health-related quality of life deterioration versus those patients treated in the placebo arm (12.7 versus 8.3 months) [26]. Thus, these findings suggested both symptomatic and quality-of-life benefit with abiraterone for asymptomatic or minimally symptomatic, chemotherapy-naïve mCRPC patients.

Subsequent pending secondary analyses include correlation of radiographic progression free survival and OS. Preliminary data suggest that these are positively linked, with a correlation coefficient of 0.7 [Ryan et al. ESMO 2012]. Further, preliminary results from another secondary analysis suggest that the most significant rPFS and OS benefits are observed in patients with a low overall burden of disease. In light of the apparent clinical heterogeneity of patients in the mCRPC clinical state, a prognostic model is currently in development.

Galeterone (TOK-001)

Galeterone is a novel C 17,20-lyase inhibitor with associated multifunctional disruption AR signaling. It directly competitively inhibits ligand binding to the AR, with concomitant down regulation of AR protein expression [27]. The ARMOR1 was a phase 1 dose-escalation study evaluating the safety, optimal dosing and efficacy of galeterone in patients with mCRPC. In general, galeterone was well tolerated, save for fatigue, weakness, and transient mild elevation in liver function tests in 15 of the 49 patients. Efficacy was evaluated by PSA response, with 11 of 49 (22 %) patients demonstrating a greater than 50 % decline in PSA from baseline, with greater PSA declines observed at higher doses [28].

The safety and efficacy of galeterone is currently under evaluation in the phase II ARMOR2 trial. This study aims to evaluate the safety and efficacy of galeterone in multiple patient populations, including patients with CRPC with or without metastatic disease, and with or without a history of failure of prior therapies including abiraterone and/or enzalutamide.

Future Directions

To date, abiraterone remains the most fully studied and developed CYP 17 inhibitor and has demonstrated clinical efficacy in the treatment of CRPC. Owing to evidence that mCRPC—and most likely CYP 17A1 inhibitor resistant prostate cancer—is a hormonally and AR-mediated state, multiple highly selective small molecule inhibitors of CYP 17A1 are currently in development. Ongoing and future clinical trials look to combine abiraterone with other AR targeted therapies. One such trial conducted through the Alliance for Clinical Trials in Oncology seeks to combine enzalutamide therapy with or without abiraterone and prednisone, with a primary objective of comparing overall survival in the study groups (NCT01949337).

Additionally, CYP 17A1 inhibition is being explored in earlier clinical settings. SWOG trial

S1216 is a phase III randomized trial comparing the combination of ADT with orteronel versus ADT and bicalutamide, with a primary objective of comparing overall and disease-free survival in the treatment arms.

The majority of these ongoing trials have focused on CYP 17A1 inhibition in patients with incurable CRPC with or without metastatic disease. A recent phase II study assessed the efficacy of abiraterone acetate plus LHRH agonist therapy in the neoadjuvant setting prior to radical prostatectomy. The combination of abiraterone plus LHRH agonist treatment was associated with a 25 % pathologic complete response rate, and was generally well tolerated—overall, toxicity was similar to that observed in trials of patients with CRPC. Although this study suggested the potential for therapeutic benefit with CYP 17A1 inhibition, prior trials of ADT in the neoadjuvant setting have not demonstrated clinical benefit. Therefore, this data supports the need for future evaluation of neoadjuvant CYP 17A1 inhibition in patients with high risk, localized disease.

Nonetheless, little is known regarding the specific mechanisms by which CYP 17A1 inhibitor resistance develops. CRPC xenografts treated with CYP 17A1 inhibitors have demonstrated relatively increased expression full-length AR, AR splice variants, and CYP 17A1. These findings suggest that resistance may develop via intratumoral upregulation of CYP 17A1 and augmented intracrine signaling, and potentially via AR splice variants capable of steroid-independent activation [6, 31]. Further, AR mutations have been identified which sensitize the AR to androgen precursors, other steroid hormones including progesterone and pregnenolone, and glucocorticoids [6, 29]. More recently, mutagenesis screens have revealed a specific AR mutation (F876L) that confers resistance to the novel AR antagonist enzalutamide, causing it instead to function as an AR agonist [30]. Through the Stand Up 2 Cancer (SU2C) initiative, tissue biopsies are being collected from patients treated with abiraterone, with an ultimate goal of clarifying those mechanisms by which CRPC tissue develops CYP 17A1 inhibition resistance.

In addition to their clinical efficacy, the development of these agents has supported the concept that many patients with this disease retain a tumor that is sensitive to manipulation of the androgen: androgen receptor axis. The optimal timing, sequence, and combination of these and similar agents continue to be evaluated; however, the arrival of these agents in the clinic in the recent years has no doubt led to significant clinical benefit for a large population of patients.

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Cytotoxic Chemotherapy (Taxanes and Taxane Combinations)

8

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Introduction

Urologists have traditionally been the primary caregivers for patients with prostate cancer and patients were referred to medical oncologists only in very late stages. With the advent of docetaxel chemotherapy, this pattern has drastically changed. This has led to better cooperation among physicians and important phase III studies which have shown a survival advantage not only with docetaxel but also with cabazitaxel chemotherapy, novel hormonal therapies, immunotherapy, and novel radiation therapy. Oncologists are highly skilled in administering chemotherapy. With more than two decades of experience with taxanes in a variety of solid tumors, in-depth knowledge and understanding of potential drug–drug interactions, dose modifications, strategies for dealing with patients with medical comorbidities, and toxicities has been attained. Since most patients remain with their medical oncologist during the later stages of their disease, post-docetaxel registrations of abiraterone and enzalutamide have in most cases been spearheaded by medical oncologists, with similar arguments about potential drug–drug interactions and handling of toxicities. Oncologists have primarily though not exclusively been involved in the drug development of novel hormonal therapies in the post-docetaxel setting. As these hormonal agents become more widely used prior to chemotherapy, both urologists and medical oncologists will most likely be more intimately involved in their administration.

The right treatment sequence and the most optimal choice for an individual patient still require further research and development. In this chapter, we have eluded to various potential predictive factors for benefit with abitaterone and with docetaxel that may impact the treatment choice. In addition, there is an increasing concern about the effectiveness of taxanes post-new generation AR inhibiting drugs. Whoever treats patients with castration-resistant prostate cancer should be encouraged to evaluate their patients in a multidisciplinary team approach.

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Mitoxantrone

In 1996 Tannock et al. reported on a phase III study involving 161 patients with metastatic castration-resistant disease who were randomized

F. Saad and M.A. Eisenberger (eds.), *Management of Castration Resistant Prostate Cancer*, Current Clinical Urology, DOI 10.1007/978-1-4939-1176-9_8,

to mitoxantrone 12 mg/m^2 every 3 weeks plus prednisone or prednisone alone [1]. Pain response was the primary endpoint and this was achieved in 29 % of patients treated with mitoxantrone, compared to 12 % of patients treated with prednisone ($p=0.01$). Despite superior pain response rates, mitoxantrone did not impact overall survival (OS) which was 12 months in both treatment arms ($p=0.27$). A trial comparing mitoxantrone plus hydrocortisone versus hydrocortisone alone was conducted by the Cancer and Leukemia Group B (CALBG) to evaluate OS. No survival benefit was observed in this study, although there was a small but significant increase in time to disease progression in the mitoxantrone arm [2]. Based upon these results, the Food and Drug Administration (FDA) approved mitoxantrone as palliative chemotherapy in patients with castration-resistant prostate cancer. Consequently, mitoxantrone became the control arm in the two pivotal phase III trials investigating docetaxel in patients with mCRPC. Anthracyclines and more specifically mitoxantrone were the standard for cytotoxic chemotherapy until the introduction of docetaxel in 2004 and treatment of men with metastatic castration-resistant prostate cancer (mCRPC) was primarily driven by symptom palliation.

Docetaxel

Microtubules are the main target of taxanes, which bind to a specific binding site on the tubulin β-subunit. Taxanes suppress microtubule dynamics by promoting tubulin assembly and stabilizing microtubules, blocking mitosis at the metaphase/anaphase transition, which results in cell death [3–5]. It has been recently shown that AR transport is facilitated by microtubules and the motor protein dynein. By interfering with microtubules, taxanes also inhibit AR nuclear transport, a known mechanism of antitumor activity in mCRPC [6–8] (Fig. 8.1).

Following phase I/II studies yielding PSA responses, pain responses, and objective tumor responses for docetaxel [9, 10], two large phase III trials TAX327 and SWOG 99-16 were initiated [11, 12]. TAX327 was conducted in 1,006 men with mCRPC who were randomized to receive 3-weekly docetaxel (75 mg/m^2), weekly docetaxel (30 mg/m^2), or 3-weekly mitoxantrone (12 mg/m^2), each with prednisone [11]. OS of patients who were treated with docetaxel in the 3-weekly regime was superior as compared to mitoxantrone with an OS of 19.2 vs. 16.3 months (HR 0.79, 95 % CI 0.67–0.93) in the final analysis [13]. The docetaxel 3-weekly arm also showed better palliation, with more patients having pain (35 vs. 22 %, $p=0.01$) and quality of life responses (22 vs. 13 %, $p=0.009$) as compared to mitoxantrone. The docetaxel weekly schedule showed a trend towards improved OS, but did not reach statistical significance. The TAX327 updated survival analysis also contained a post-hoc analysis which demonstrated that the trends in OS were consistent among several subgroups of patients based on age (<68 vs. ≥68 years), pain vs. no pain at baseline, and baseline PSA <115 vs. ≥115 ng/mL.

Neutropenia was the most common observed grade 3/4 toxicity and occurred more frequently in patients receiving 3-weekly docetaxel (32 %). Despite the high incidence of neutropenia, febrile neutropenia was rare (3 %) and other grade 3/4 toxicities all occurred in less than 5 %.

A second trial, SWOG 99-16 was designed on the assumption that the combination of docetaxel and estramustine had the greatest therapeutic potential. Seven-hundred and seventy patients were randomized to receive 280 mg estramustine three times daily on days 1–5, plus docetaxel 60 mg/m^2 on day 2, preceded by 60 mg of dexamethasone divided in three doses, or mitoxantrone 12 mg/m^2 on day 1 plus 5 mg of prednisone twice daily [12]. Both were given in a 21-day cycle, and dose escalation to docetaxel 70 mg/m^2 or mitoxantrone 14 mg/m^2 was allowed in cycle 2 if no grade 3/4 toxicities were observed during the first cycle. Median OS was superior in the group receiving docetaxel as compared to mitoxantrone (17.5 vs. 15.6 months, respectively), with an HR of 0.80 (95 % CI 0.67–0.97). The group treated with docetaxel and estramustine

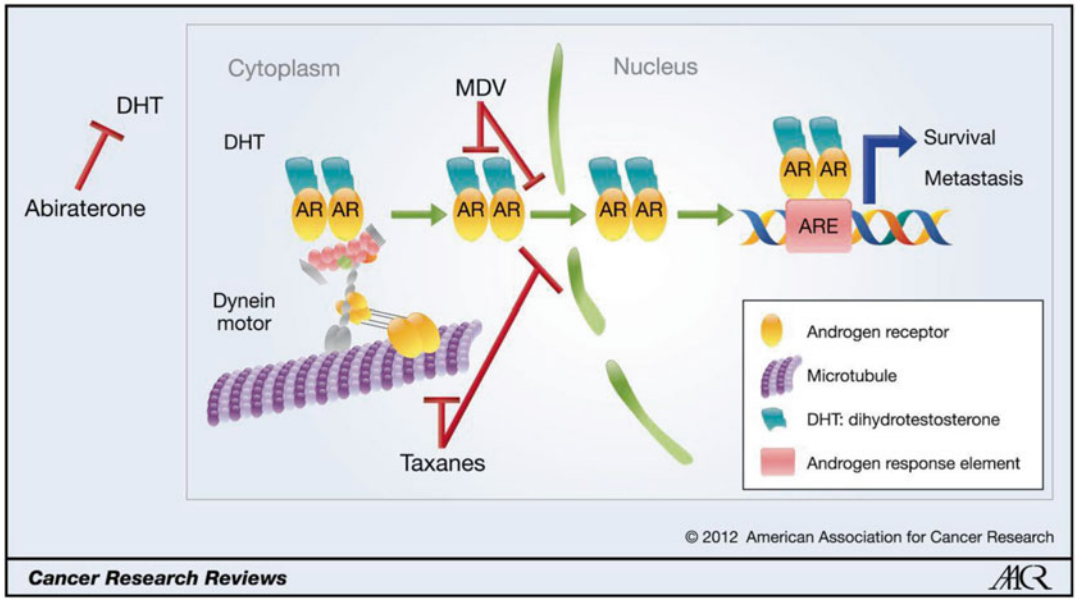

Fig. 8.1 Proposed model of taxane mechanism of action in prostate cancer. AR associates with microtubules and translocates to the nucleus via the motor protein dynein. Taxanes inhibit depolymerization of microtubules and block microtubules dynamics. By interfering with microtubule dynamics, taxanes cause a cell cycle arrest in the G2/M phase, and inhibit AR nuclear translocation as an additional mechanism of action in mCRPC. The mechanisms of action of enzalutamide (MDV3100) and abiraterone are also shown. Enzalutamide exerts its effect by inhibiting AR nuclear translocation, DNA-binding and co-activator recruitment. Abiraterone inhibits androgen biosynthesis by irreversibly blocking CYP17A1, a crucial enzyme in steroidogenesis. Reprinted by permission from the American Association for Cancer Research: Thadani-Mulero M. Androgen receptor on the move: boarding the microtubule expressway to the nucleus. Cancer Research. 2012;72(18):4611–5

had significantly higher rates of grade 3 and 4 neutropenic fever (5 vs. 2 %), cardiovascular events (15 vs. 7 %), and nausea and vomiting (20 vs. 5 %), as compared with the group treated with mitoxantrone and prednisone.

Taken together, the results of these two phase III studies showed that docetaxel in a 3-weekly regimen improved OS, which was the primary endpoint of both trials. Weekly docetaxel did not appear to be better tolerated than the 3-weekly regimen, and showed only a trend towards better efficacy as compared to mitoxantrone. The SWOG study did not reveal greater benefit by the addition of estramustine. Because of the lack of superior activity and greater toxicity by the addition of estramustine, docetaxel every 3 weeks plus low-dose prednisone subsequently became the standard of care for patients with mCRPC [14].

In multivariate analysis of TAX327, a total of ten independent prognostic factors for survival were identified including the presence of liver metastases, number of metastatic sites, clinically significant pain, Karnofsky performance status, type of progression, pretreatment PSA doubling time, baseline PSA, tumor grade, baseline alkaline phosphatase, and baseline hemoglobin [15]. These prognostic factors have been elaborated into a nomogram (Fig. 8.2). Such decision making tools are informative, can facilitate tailoring of therapy, and can simplify important clinical decisions such as when to start cytotoxic chemotherapy. Although the survival benefit obtained by docetaxel compared with mitoxantrone is consistent among patients with and without pain at baseline (HR 0.73 and 0.85, respectively), there is a substantial difference

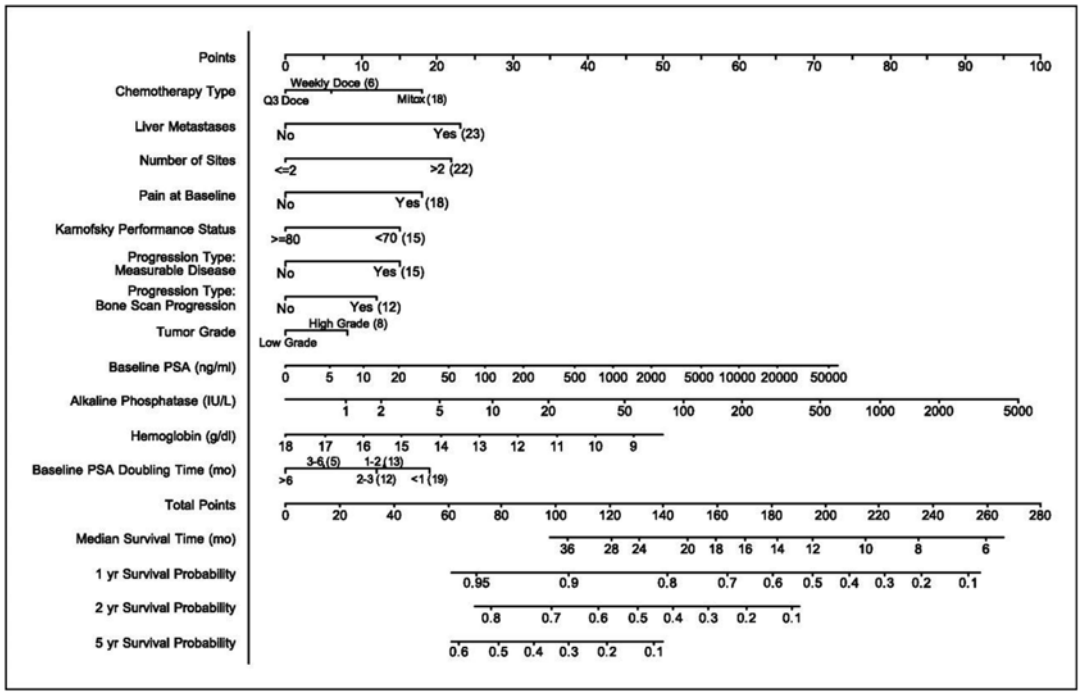

Fig. 8.2 Nomogram for survival of patients with progressive mCRPC, including data derived from 686 patients and 518 mortality events. *Note*: A present pain intensity of ≥2 and/or an analgesic score of ≥10 were defined in the original protocol as indicative of the presence of significant pain. Instructions for physician: Locate the liver metastasis axis. Draw a straight line upward to the points axis to determine how many points toward survival the patient receives for the presence or absence of liver metastases. Repeat this process for each predictor variable and sum the points for each predictor. Locate this sum on the total points axis. Draw a straight line downward from the total points axis to identify the predicted median survival and the predicted 1-, 2-, and 5-years predicted overall survival probabilities. Instructions to patient: "Mr. X, if we had 100 men exactly like you, we would expect < nomogram prediction × 100 > to be alive in 1, 2, and 5 years, respectively, and we expect 50 of those to be alive after < median survival prediction > months." Reprinted by permission from the American Association for Cancer Research: Armstrong A. A contemporary prognostic nomogram for men with hormone-refractory metastatic prostate cancer: a TAX327 study analysis. Clinical Cancer Research. 2007;13(21):6396–403

in OS time (14.4 months for patients with pain vs. 21.3 months for patients without pain). However, this does not necessarily imply benefit from the early use of chemotherapy, but may rather guide treatment in asymptomatic patients by defining patients at greater risk of imminent disease progression and death. These patients may be candidates for chemotherapy, even in the absence of symptoms.

Nonetheless, in the TAX327 study a decrease in quality of life was more often observed in patients with minimal symptoms at the start of chemotherapy [16]. Therefore delaying chemotherapy may be a suitable approach in patients with minimal symptoms. Those patients who have no symptoms yet, but are more likely to develop symptoms in the near future due to bone scan progression and/or the development of anemia should be considered candidates for docetaxel chemotherapy [14].

With the recent FDA and EMA approval of the CYP17 inhibiting agent abiraterone in the pre-docetaxel setting, it has become increasingly important to identify subgroups of patients who may have greater benefit by the use of chemotherapy in order to better tailor treatment choices. Recently, Azria et al. reported a high Gleason score [8–10] at the time of diagnosis to be an

independent risk factor for poor response to abiraterone [17, 18]. In addition, a retrospective analysis of patients with mCRPC enrolled in clinical trials demonstrated that patients who had a short response to prior androgen deprivation therapy (ADT) (<16 months) had poor PSA responses and PFS when treated with secondary hormonal therapies such as abiraterone and enzalutamide [19]. In this light, a recent post-hoc analysis of the TAX327 study was conducted which revealed that the survival benefit obtained with docetaxel as compared to mitoxantrone was most pronounced in patients with high Gleason score tumors (Gleason 7–10) [20]. Furthermore, two prospective databases of patients with mCRPC demonstrated similar PSA responses and clinical benefit obtained by docetaxel, irrespective of the duration of response to ADT [21].

In an era of shifting paradigms in mCRPC with abiraterone becoming available also prior to docetaxel chemotherapy, Gleason score and prior response to ADT may serve to discriminate between patients who benefit most from docetaxel chemotherapy as first-line treatment. Docetaxel seems to exert efficacy particularly in high Gleason score tumors irrespective of response to ADT. In contrast, in patients with better differentiated tumors and durable responses to ADT abiraterone might be a good treatment option. In the future, these observations should be prospectively validated in order to further personalize first-line treatment options for patients with mCRPC.

Mechanism of Action of Taxanes: Emerging Data on AR Transport as Part of Their Efficacy

As mentioned above, docetaxel and cabazitaxel also impair AR signaling, which in the setting of mCRPC might in fact be responsible for part of the therapeutic efficacy [6–8]. Recently, clinical and preclinical evidence is emerging about potential cross-resistance between docetaxel and abiraterone [8]. A clinical report on patients treated with docetaxel who had previously been treated with abiraterone showed an OS of only 12.5 months, which was significantly less than the 19 months predicted for this patient population [22]. Moreover, PSA declines ≥50 % were observed in 26 % of patients, compared to 45 % in the TAX327 study [11], and no responses to docetaxel were observed in abiraterone-refractory patients. A likely explanation is that antitumor activity of taxanes in mCRPC is partly depending on its impact on AR signaling. When patients are treated with abiraterone first, it could very well result in impaired effectiveness of docetaxel due to annulling its effects on the AR. Hence the sequence of abiraterone followed by docetaxel upon progression could result in decreased effectiveness of the chemotherapy and thus impair the eventual clinical benefit.

Cabazitaxel, however, seems to retain activity in the third-line setting following docetaxel and abiraterone, with ≥50 % PSA declines in 42–49 % of patients [23, 24]. Prospective clinical studies should further define the implications for the optimal treatment sequence of these treatment options for patients with mCRPC.

Docetaxel Retreatment

Sooner or later all patients will progress during or after treatment with docetaxel. Patients who relapse after an initial response to docetaxel may again respond to a second or even third series of docetaxel cycles [25–27]. Since the phase II data on docetaxel rechallenge have been limited to efficacy, i.e. PSA responses, pain responses, and objective responses and data on survival benefit are lacking, such rechallenge has become a less likely choice following the introduction of the new agents such as cabazitaxel, abiraterone, and enzalutamide, which have all demonstrated survival benefit in patients relapsing after docetaxel chemotherapy [28–30].

An alternative approach to the standard of 10–12 cycles docetaxel as used in the pivotal phase III studies is intermittent dosing of docetaxel

suspending treatment after six cycles or at a predefined PSA decrease, and retreatment when PSA starts to rise again. In one of the larger studies a majority of patients responded again to such retreatment [31]. These data are of particular interest because of the absence of a defined optimal duration of chemotherapy in responding cases with mCRPC [26, 32]. In a prospective phase II study, patients were enrolled who had responded to first-line docetaxel and progressed after a chemotherapy-free interval of at least 5 months. Median overall survival since enrollment was 13 months, and a 50 % PSA decline was observed in 24.5 % of patients [25]. Like for docetaxel retreatment, OS data for intermittent docetaxel therapy are also lacking, and a second series of docetaxel has become questionable due to the newly available systemic treatment options.

Docetaxel-Based Combination Therapies

In the light of improved survival and modest toxicity with docetaxel as was demonstrated in TAX 327, numerous investigators, collaborative groups, and industry have investigated whether the efficacy of docetaxel could be improved by adding a second agent [33]. Here we will discuss docetaxel combination studies. An overview of the phase III combination trials with docetaxel is shown in Table 8.1.

Table 8.1 Phase III trials of docetaxel-based combinations

Agent	Result
Docetaxel + GVAX (VITAL-2)	OS inferior in combination arm: 12.2 vs. 14.1 months HR 1.70 (95 % CI 1.15–2.53)
Docetaxel + Calcitriol (ASCENT-2)	OS inferior in combination arm: 17.8 vs. 20.2 months HR 1.42 (95 % CI 1.13–1.86)
Docetaxel + Atrasentan (SWOG S0421)	OS not improved in combination arm: 18 vs. 17 months HR 1.01 (95 % CI 0.87–1.18)
Docetaxel + Zibotentan (ENTHUSE M1C)	OS not improved in combination arm: 20 vs. 19.2 months HR 1.00 (95 % CI 0.84–1.18)
Docetaxel + Lenalidomide (MAINSAIL)	OS inferior in combination arm: 17.7 vs. median not reached HR 1.53 (95 % CI 1.17–2.00)
Docetaxel + Bevacizumab (CALBG 90401)	OS not improved in combination arm: 22.6 vs. 21.5 months HR 0.91 (95 % CI 0.78–1.05)
Docetaxel + Aflibercept (VENICE)	OS not improved in combination arm: 22.1 vs. 21.2 months HR 0.94 (95.6 % CI 0.82–1.08)
Docetaxel + Dasatinib (READY)	OS not improved in combination arm: 21.5 vs. 21.2 months HR 0.99 (95 % CI 0.87–1.13)
Docetaxel + Custirsen (SYNERGY)	Ongoing

Phase III Trials

Immunotherapy

The GVAX platform of immunotherapies involved injection of cells derived from prostate cancer cell lines to provoke an immune response to multiple antigens expressed by the tumor cell. In addition, the cells were modified to secrete granulocyte macrophage colony-stimulating factor (GM-CSF). The VITAL-2 trial compared GVAX plus 3-weekly docetaxel with docetaxel plus prednisone and was interrupted early due to an unexpected higher death rate in the GVAX arm (67 deaths for GVAX plus docetaxel vs. 47 deaths for docetaxel plus prednisone) [34]. Another trial (VITAL-1) compared GVAX with docetaxel in patients with asymptomatic CRPC [35]. The study was prematurely terminated based on the results of a futility analysis conducted by the study's Independent Data Monitoring Committee (IDMC) which determined that the study had less than a 30 % chance of meeting its predefined primary endpoint of improvement in overall survival.

Calcitriol

Calcitriol is an activated vitamin D analog that has shown to enhance antitumor activity of paclitaxel and docetaxel in vitro and in vivo [36, 37]. ASCENT-1 was a double-blind randomized phase II study that investigated weekly docetaxel plus high-dose calcitriol versus docetaxel plus placebo [38]. The primary endpoint PSA response rate did not differ between the treatment groups. Although it was not the primary endpoint of the trial, there was an improvement in OS for calcitriol over the placebo group. The ASCENT-2 trial was a randomized phase III trial designed to validate the observed survival benefit obtained with docetaxel plus calcitriol in the ASCENT trial [39]. In the phase III trial the control arm comprised the standard docetaxel regimen every 3 weeks. At an interim analysis, more deaths were noted in the ASCENT arm and consequently the trial was terminated early. Median OS was 17.8 months (95 % CI 16.0–19.5) for docetaxel plus calcitriol compared to 20.2 months (95 % CI 18.8–23.0) for docetaxel plus prednisone. Reasons for the worse OS by docetaxel plus calcitriol arm may have been attributed to the use of the weekly docetaxel schedule in the investigational arm [39].

Endothelin-A Receptor Antagonists

Atrasentan is an endothelin-A receptor antagonist that enhanced the effects of docetaxel against prostate cancer cells in vitro and in vivo [40, 41]. In the SWOG S0421 trial atrasentan plus docetaxel and prednisone was investigated in 991 patients with bone metastases. No difference in OS and PFS was observed for atrasentan plus docetaxel and prednisone compared with docetaxel and prednisone alone [42].

Another endothelin-A receptor antagonist zibotentan was investigated in the phase III trial ENTHUSE M1C combined with standard docetaxel versus docetaxel plus placebo. Docetaxel plus zibotentan did not result in a significant improvement in OS compared with docetaxel plus placebo (HR 1.00, 95 % CI 0.84–1.18) [43].

Angiogenesis Inhibitors

The oral angiogenesis inhibitor thalidomide demonstrated additive effects to taxane chemotherapy in vitro [44]. In two randomized phase II trials, the addition of thalidomide to docetaxel resulted in an encouraging PSA decline rates. Although more thromboembolic events were observed in patients treated with thalidomide, the combination regimen was reported to be well tolerated after the administration of prophylactic low-molecular-weight heparin [45, 46]. Lenalidomide is the successor of thalidomide with greater anti-angiogenesis efficacy as well as immunomodulatory effects. The randomized phase III trial (MAINSAIL) evaluated the efficacy of lenalidomide plus docetaxel versus docetaxel and placebo as first-line treatment for mCRPC. Following an interim analysis the study was stopped due to greater toxicity in the investigational arm and possibly reduced effectiveness. This could have been due to more frequent docetaxel dose reductions in patients allocated to lenalidomide [47].

Bevacizumab is a humanized immunoglobulin G monoclonal antibody to all the isoforms of VEGF-A. The CALBG Group investigated the addition of bevacizumab to standard docetaxel and prednisone in a randomized phase III trial. Despite an improvement in PFS and objective response, the addition of bevacizumab to docetaxel and prednisone did not improve OS and was associated with greater toxicity [48].

Aflibercept, a recombinant human fusion protein that binds A and B isoforms of VEGF and placental growth factor thereby inhibiting angiogenesis, was investigated in the phase III VENICE trial. In this study 1,224 men were treated with docetaxel plus prednisone and randomized to receive aflibercept or placebo. Median overall survival was 22.1 months (95.6 % CI 20.3–24.1) in the aflibercept group and 21.2 months (95.6 % CI 19.6–23.8) in the placebo group (stratified hazard ratio 0.94; 95.6 % CI 0.82–1.08; $p = 0.38$). The combination of aflibercept and docetaxel was associated with a higher incidence of grade 3/4 gastrointestinal disorders, hemorrhagic events, hypertension, fatigue, infections, and treatment-related fatal adverse events [49].

Bone Microenvironment Agents

SRC-family kinases play an important role in prostate cancer growth and invasion, as well as the pathogenesis of bone metastases and the regulation of osteoclast function [50–52]. Among others, dasatinib potently inhibits the SRC-family kinases (SRC, LCK, HCK, FYN, YES, FGR, BLK, LYN, and FRK [53]). In preclinical studies the tyrosine kinase inhibitor dasatinib inhibited cell duplication, migration, and invasion, and triggered apoptosis of tumoral cells. Dasatinib also acts on the tumor microenvironment, which is particularly important in the bone, where it inhibits osteoclastic activity and favors osteogenesis, exerting a bone-protecting effect [53]. These preclinical studies led to the hypothesis that combining dasatinib with docetaxel would improve treatment outcomes by targeting both the tumor and bone microenvironment. In a phase I/II study combining docetaxel with dasatinib, 18 out of 30 patients with measurable disease had a partial response and 14 patients had disappearance of lesions on bone scans [54]. However, the phase III READY trial demonstrated no survival benefit for docetaxel plus dasatinib compared to docetaxel and placebo [55].

Custirsen

Clusterin (CLU) is a stress-activated cytoprotective chaperone upregulated by a variety of anticancer therapies that lends treatment resistance when overexpressed [56]. Preclinical studies have shown that knockdown of clusterin enhances the effects of docetaxel in docetaxel-refractory cells [57]. A randomized phase II trial investigated custirsen (OGX-11), an antisense inhibitor of clusterin, in combination with docetaxel and prednisone, versus docetaxel and prednisone alone. The combination of docetaxel and prednisone with OGX-11 was associated with a longer median OS, despite similar rates of PSA and tumor response [58]. Two phase III trials of OGX-11 in first- and second-line treatment of mCRPC are currently underway. Due to its unique mechanism of action, these trials are the only ongoing phase III studies with the potential of having a positive outcome in terms of survival benefit.

Phase II Trials

The addition of bcl-2 inhibitor AT-101 in combination with docetaxel was evaluated in a phase II trial with OS as the primary endpoint. The addition of AT-101 did not extend OS, PFS, or PSA response as compared with docetaxel and prednisone [59]. The bcl-2 antisense oligonucleotide oblimersen was combined with docetaxel in an EORTC phase II trial. Primary endpoints including a rate of confirmed PSA response >30 % and a major toxic event rate <45 % were not reached [60].

In a randomized phase II trial of docetaxel and vandetanib, an oral inhibitor of vascular endothelial growth factor receptor (VEGFR) and epidermal growth factor receptor (EGFR), no benefit was reported for the combination compared to docetaxel and placebo [61]. A single-arm phase I/II trial of docetaxel plus sunitinib, an inhibitor of VEGFR and platelet derived growth factor (PDGFR) demonstrated PSA responses in 56.4 % of patients [62]. In another single-arm phase II trial evaluating sorafenib and docetaxel PSA responses were observed in 46 % of patients [63].

The PDGFR inhibitor imatinib was also investigated in combination with weekly docetaxel in a phase II trial. Increased adverse gastrointestinal events were observed in the experimental arm. These events coupled with a futility analysis which indicated that a significant treatment difference would be unlikely for the planned accrual of 144 patients, led to early termination of the study [64].

None of these agents is currently under investigation in a phase III clinical trial.

In summary, docetaxel plus prednisone remains the gold standard of chemotherapy. None of the eight phase III docetaxel-based combination trials have demonstrated a survival benefit when compared with the standard docetaxel regimen. A critical assessment by Antonarakis and Eisenberger of the phase II trials that led to the initiation of these studies showed that the results might not have been sufficient for the conduction of large phase III studies. Either no phase II data

were available, or the metric for success that would prompt phase III development was not defined or reached [65].

Cabazitaxel

Cabazitaxel was selected from 450 taxane derivatives, based on its antitumor activity in docetaxel-resistant tumor models [5]. Unlike the other taxanes (paclitaxel and docetaxel), cabazitaxel has poor affinity for the drug transporter p-glycoprotein (P-gp, ABCB1) [66, 67]. An additional characteristic of cabazitaxel is its ability to penetrate the blood–brain barrier in vivo, which is limited with other taxanes [68]. Recently it has been demonstrated that cabazitaxel also inhibits AR nuclear translocation, which could be an additional mechanism of taxane antitumor activity in mCRPC [8].

A phase I trial in patients with solid tumors determined that cabazitaxel had linear pharmacokinetics similar to docetaxel, but probably better tolerability [66]. The principal dose-limiting toxicity was neutropenia, with one patient experiencing febrile neutropenia and two others showing prolonged grade 4 neutropenia at the 25 mg/m^2 dose level. Non-hematologic toxicities included nausea, vomiting, diarrhea, neurotoxicity, and fatigue, and were generally mild to moderate. Objective antitumor activity was observed in two patients with partial responses including one patient with docetaxel-refractory mCRPC. One patient had an unconfirmed partial response and two patients had minor responses. Subsequently, two proof of principle trials were conducted which demonstrated responses in patients with taxane resistant metastatic breast cancer [67, 69].

The Phase III TROPIC trial was a randomized, open-label, multicenter trial, conducted in 755 men with mCRPC who progressed during or after docetaxel chemotherapy [29]. Patients were randomized to receive either cabazitaxel 25 mg/m^2 or mitoxantrone 12 mg/m^2 in a 3-weekly regimen, each with 10 mg prednisone daily. Median OS was 15.1 months for the cabazitaxel arm versus 12.7 months in the mitoxantrone arm, with a hazard ratio (HR) for death of 0.70 (95 % CI 0.59–0.83, $p<0.0001$). Secondary endpoints including progression free survival, PSA response, objective tumor response according to RECIST criteria, time to PSA progression, and median time to tumor progression were all significantly improved in the cabazitaxel arm. Pain response rates were similar between the two treatment arms.

About 70 % of patients had progressive disease during or within 3 months after docetaxel treatment, including about 30 % of patients who had disease progression during docetaxel treatment. The benefit of cabazitaxel as compared to mitoxantrone was consistent among subgroups of patients defined by prognostic factors including patients with disease progression during docetaxel treatment and in those who received high cumulative doses of docetaxel.

In concordance with the TAX327 trial, a post-hoc analysis of the TROPIC trial linked a significant OS benefit for cabazitaxel versus mitoxantrone to patients with poorly differentiated tumors evaluated by WHO grade (median OS 15.2 vs. 12.7 months, $p<0.0001$), whereas for patients with well or moderately differentiated tumors this benefit was less robust, with a median OS of 15.5 months for cabazitaxel and 13.3 months for mitoxantrone ($p=0.56$) [70]. In this post-hoc analysis, the OS benefit obtained by cabazitaxel was independent of the duration of ADT. In contrast, a high Gleason score (Gleason 8–10) and a short response to prior ADT ($\leq$16 months) may be predictive of a poor PSA response and PFS in patients treated with abiraterone [17, 19]. These easily available parameters could potentially be of value in determining which treatment, cabazitaxel, or an agent like abiraterone has the greatest therapeutic potential in an individual patient as second-line treatment for mCRPC.

Patients received a median of six cycles for cabazitaxel, and four cycles for mitoxantrone. The most frequent hematological AEs were hematologic. Grade $\geq$3 neutropenia was more common in patients who received cabazitaxel (82 %) than in patients who received mitoxantrone (58 %), with febrile neutropenia rates of 8 and 1 %,

respectively. The most frequent non-hematologic AE was diarrhea, occurring in 47 % (grade ≥3, 6 %) of patients treated with cabazitaxel, compared to 11 % (grade ≥3, <1 %) of patients treated with mitoxantrone.

A total of 18 patients (4.9 %) who were treated with cabazitaxel died from causes other than disease progression within 30 days of receiving their last dose of cabazitaxel. This compares with three drug-related patient deaths (0.9 %) in the mitoxantrone group. The most common cause of death in patients who were treated with cabazitaxel was neutropenia and its clinical consequences. However, no further deaths due to neutropenic complications occurred in the cabazitaxel group following the IDMC communication to the TROPIC investigators about the need to strictly adhere to the study protocol regarding dose delays and modifications and to manage neutropenia with granulocyte colony-stimulating factor (G-CSF) according to American Society for Clinical Oncology (ASCO) guidelines. The frequency of hematological adverse events and related deaths demonstrates that cabazitaxel treatment requires careful monitoring and management of emerging symptoms. Dose reductions as well as the administration of G-CSF according to ASCO guidelines are strategies that should be considered in patients with high risk clinical features (age ≥65 years, poor performance status, previous episodes of febrile neutropenia, extensive prior radiation ports, poor nutritional status, or other serious comorbidities) to manage side effects of treatment with cabazitaxel. In an attempt to reduce cabazitaxel induced toxicity, an open-label randomized phase II study (CABARESC) is currently testing whether the addition of the oral poorly resorbable steroid budesonide reduces or protects against cabazitaxel induced diarrhea.

FIRSTANA is a randomized phase III trial with OS as the primary endpoint comparing cabazitaxel 25 mg/m^2 and cabazitaxel 20 mg/m^2 both with prednisone, to docetaxel 75 mg/m^2 plus prednisone as first-line treatment for mCRPC. PROSELICA is an ongoing trial with a non-inferiority design comparing cabazitaxel 25 mg/m^2 to cabazitaxel 20 mg/m^2 both with prednisone. These studies will answer the questions whether a reduced dose of cabazitaxel may provide similar OS with the benefit of reduced toxicity, and whether cabazitaxel has greater therapeutic potential compared to docetaxel as first-line treatment for mCRPC.

At the present time, cabazitaxel has demonstrated survival benefit in patients progressing during or after treatment with docetaxel. In the Phase III TROPIC trial, the OS benefit obtained was consistent among the two thirds of patients enrolled who had either disease progression during docetaxel (29 %) or within 3 months after the last docetaxel cycle (45 %). Cabazitaxel has thus a different mode of action and is an important contribution to the management of patients with mCRPC who have failed docetaxel chemotherapy.

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The Emerging Role of Immunotherapy in Castrate-Resistant Prostate Cancer

9

Romualdo Barroso-Sousa and Charles G. Drake

Introduction

In the past several years, two immunotherapy agents have been granted FDA approval for cancer treatment. These include Ipilimumab, a monoclonal antibody that blocks the immune checkpoint molecule CTLA-4 in metastatic melanoma [1], and Sipuleucel-T, an autologous, cell-based vaccine for metastatic castration-resistant prostate cancer (mCRPC) [2]. These approvals, as well as exciting clinical data showing objective responses when a second immune checkpoint (PD-1) is blocked in multiple tumor types [3], have led to renewed interest in cancer immunotherapy, with a number of trials ongoing. In this chapter, we will first briefly discuss the basic biology of prostate cancer (PC), focusing on issues related to immunotherapy. Next, we will introduce the major immunotherapy platforms that have advanced to later stage clinical trials, with an emphasis on the immunological mechanism of action (MOA) of each agent. Finally, we will discuss the concept of combining immunotherapy with other treatment modalities, an approach that has already shown promise in other tumor types.

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The Immunological Characteristics of Prostate Cancer

With the exception of certain virally mediated tumors that are more prevalent in immunocompromised individuals, most human cancers develop in immunologically intact hosts. So, as tumorigenesis proceeds from low-grade/localized disease to metastasis, an interaction between the host immune system and the tumor mass occurs. This process has been reasonably well characterized in animal models, and may be divided into three distinct stages [4]. In the first stage of the process, early tumors may be recognized by the immune system in a productive, pro-active way, leading to *elimination* of small, clinically undetectable masses. Elimination is most likely mediated by a concerted effort between the innate (macrophages and dendritic cells) and adaptive (T and B cells) immune systems. As tumors progress, they acquire alterations that render an anti-tumor immune response less efficacious. Thus, in the second phase of tumor/immune system interactions, tumors are able to exist in a sort of *equilibrium* with the host immune response, with progression slowed by an ongoing immune response, but in which tumors can no longer be

F. Saad and M.A. Eisenberger (eds.), *Management of Castration Resistant Prostate Cancer*, Current Clinical Urology, DOI 10.1007/978-1-4939-1176-9_9,

successfully eliminated. Equilibrium may persist for a significant period of time, and some tumors may remain at the equilibrium stage for the life of the host. Eventually, however, some tumors proceed to *escape* the host immune response, and become clinically apparent. The molecular mechanisms involved in the escape phase are likely multiple, and often include down-regulation of tumor antigens against which a host response is directed [5]. Together, the three phases of tumor/host interactions (Elimination, Equilibrium, and Escape) collectively form the "immune editing hypothesis," which serves as a valuable framework through which to understand the immune response to cancer. Indeed, subversion of a productive host anti-tumor response is now listed as one of the hallmarks of cancer [6].

While the immune editing hypothesis would leave one with the impression that anti-tumor immune responses are generally beneficial, those data need to be considered along with a great deal of apparently contradictory data suggesting that inflammation can promote tumor progression [7]. Interestingly, human and animal studies indicate that inflammation might have a role in the development of prostate cancer (PC) [8], as well as in the progression from organ-confined to metastatic disease [9, 10]. In fact, even before the appearance of clinical symptoms, the PC tumor microenvironment is frequently infiltrated by several types of inflammatory cells including innate cells like macrophages as well as adaptive cells such as T and B cells [11]. Together, these cells orchestrate an inflammatory environment that may function to either stimulate or inhibit cancer growth. In terms of the beneficial versus pro-tumorigenic effects of each component, one very basic principle is that the innate elements of inflammation are generally pro-tumorigenic, while adaptive elements are often anti-tumor. Thus, type II macrophages and myeloid-derived suppressor cells (MDSC) drive pro-carcinogenic inflammation [12], while adaptive responses mediated by CD8 effector cells are often credited with anti-tumor efficacy. Multiple exceptions exist, the CD4 T cells that infiltrate the prostate gland are enriched for cells that turn off other adaptive immune responses (Treg) or are skewed to secrete the cytokine IL-17 [13] which is associated with tumor progression in many models [14]. Taken together, these data suggest that immune approaches to prostate cancer should not be viewed in isolation; instead immunotherapy for prostate cancer is administered to patients who likely have a complex, ongoing immune response to their tumor.

Prostate Cancer Vaccines

Given the data above, the goal of a cancer vaccine is to either induce or expand an adaptive immune response to a patient's tumor. In infectious diseases, an adaptive immune response can often be generated with fairly simple technology, by admixing pathogen-specific protein(s) with an agent designed to stimulate the host response, i.e. an adjuvant. In this regard, there is a fundamental difference between cancer vaccines and those used for infectious diseases, in that the latter are nearly always administered to un-infected patients. Thus, the vaccine proteins represent novel antigens in the host to which pre-existing tolerance has not developed. In the case of cancer vaccines, the host has often already been exposed to the cancer-associated proteins (antigens) and is therefore less likely to react. Traditional vaccines (protein + adjuvant) do not directly activate T cells from the adaptive immune system. Instead, the injected proteins are taken up by cells from the host's innate immune known as dendritic cells, which then in turn activate specific T cells [15]. Among the many cancer types that can be targeted by vaccination, there are several features of prostate cancer that render it a fairly favorable target (Table 9.1).

Sipuleucel-T

As discussed above, adaptive immune responses are primarily mediated by dendritic cells (DC), and a cancer vaccine, like any other vaccine, would be expected to depend on the presence of a population of host DC that are numerically and functionally intact. Unfortunately, this is often

Table 9.1 Prostate cancer as a vaccine target

• Prostate cancer cells usually grow more slowly than many other malignancies, thus allowing time for the elicitation of effective immune responses able to translate into clinical benefit
• Biochemically recurrent prostate cancer provides an unique opportunity for immunological intervention, as the multiple immunosuppressive mechanisms associated with an advanced tumor burden are expected to be at a minimum at this stage
• Prostate cells express many a number of specific proteins that could act as immune therapeutic targets, including prostate-specific antigen (PSA), prostatic acid phosphatase (PAP), and prostate-specific membrane antigen (PSMA)

not the case in cancer patients, in which DC are often dysfunctional [16]. One approach to overcoming this dysfunction, then, might be to generate new autologous DC outside of the patient's tolerogenic environment. This is indeed the approach involved in the generation of Sipuleucel-T, which was the first cancer vaccine approved by the FDA for the treatment (rather than prevention) of cancer. The product is individually manufactured for each patient with PC, in a process which comprises multiple steps (Fig. 9.1a). Briefly, patients undergo a standard leukopheresis procedure and peripheral blood mononuclear cells (PBMC) are separated and then incubated with PAP2024, a fusion protein that links the antigen PAP to the granulocyte-macrophage colony-stimulating factor (GM-CSF). After approximately 36 h of incubation, cells are washed and suspended in Lactate Ringer Injection, for infusion back into the patient. In this approach, the GM-CSF serves as the adjuvant that helps to activate dendritic cells and other cells in the infusion product. The process is repeated three times at 2-week intervals [17].

Clinical Data (Sipuleucel-T)

After a series of phase I and II trials, two relatively small randomized, double-blind, placebo-controlled phase III trials (D9901 and D9902A) showed evidence for the clinical activity of Sipuleucel-T [18]. Both studies enrolled men with asymptomatic or minimally symptomatic mCRPC, and both suggested clinical activity via an increased survival. Thus, the multicenter, randomized, double-blind, placebo-controlled IMPACT trial was designed with primary endpoint of OS. trial trail enrolled 512 patients with asymptomatic or minimally symptomatic mCRPC randomized 2:1 to receive Sipuleucel-T or placebo. Resulting data showed that treatment with Sipuleucel-T was increased median OS by 4.1 months (25.8 versus 21.7 months; $p = 0.032$, HR = 0.78) [19]. As in the prior phase III studies, median time to progression was not different between groups, and radiographic responses were not generally observed. Sipuleucel-T was generally well tolerated, and 92 % of patients received all three infusions. The most common adverse events (AEs) in the Sipuleucel-T group included chills (in 51.2 %), fever (22.5 %), fatigue (16.0 %), nausea (14.2 %), and headache (10.7 %); all these were graded as mild or moderate. Adverse events of grade 3 or more within 1 day after infusion were rare, and were reported in 23 of 338 patients (6.8 %) in the Sipuleucel-T group and 3 of 168 patients (1.8 %) in the placebo group.

Mechanism of Action

The final Sipuleucel-T product is heterogeneous, comprising mature antigen-presenting cells (APC) and other cell types, including T cells, B cells, and natural killer cells [17]. Once infused, the autologous ex vivo-activated APC are thought to prime PAP-specific CD4+ and CD8+ T cells in a manner similar to a classical vaccine-mediated prime-boost regimen, where the first infusion primes the immune system and subsequent infusions boost the response [20]. Recently, a combined analysis of immunological data from the previously cited phase III trials (D9901, D9902A, and IMPACT) was completed [21]. The authors demonstrated that APC activation in the infused product occurred with the initial dose, and increased with subsequent doses. The median cumulative APC activation with Sipuleucel-T across the three dose preparations was 26.7 [95 % CI 21.5–33.6].

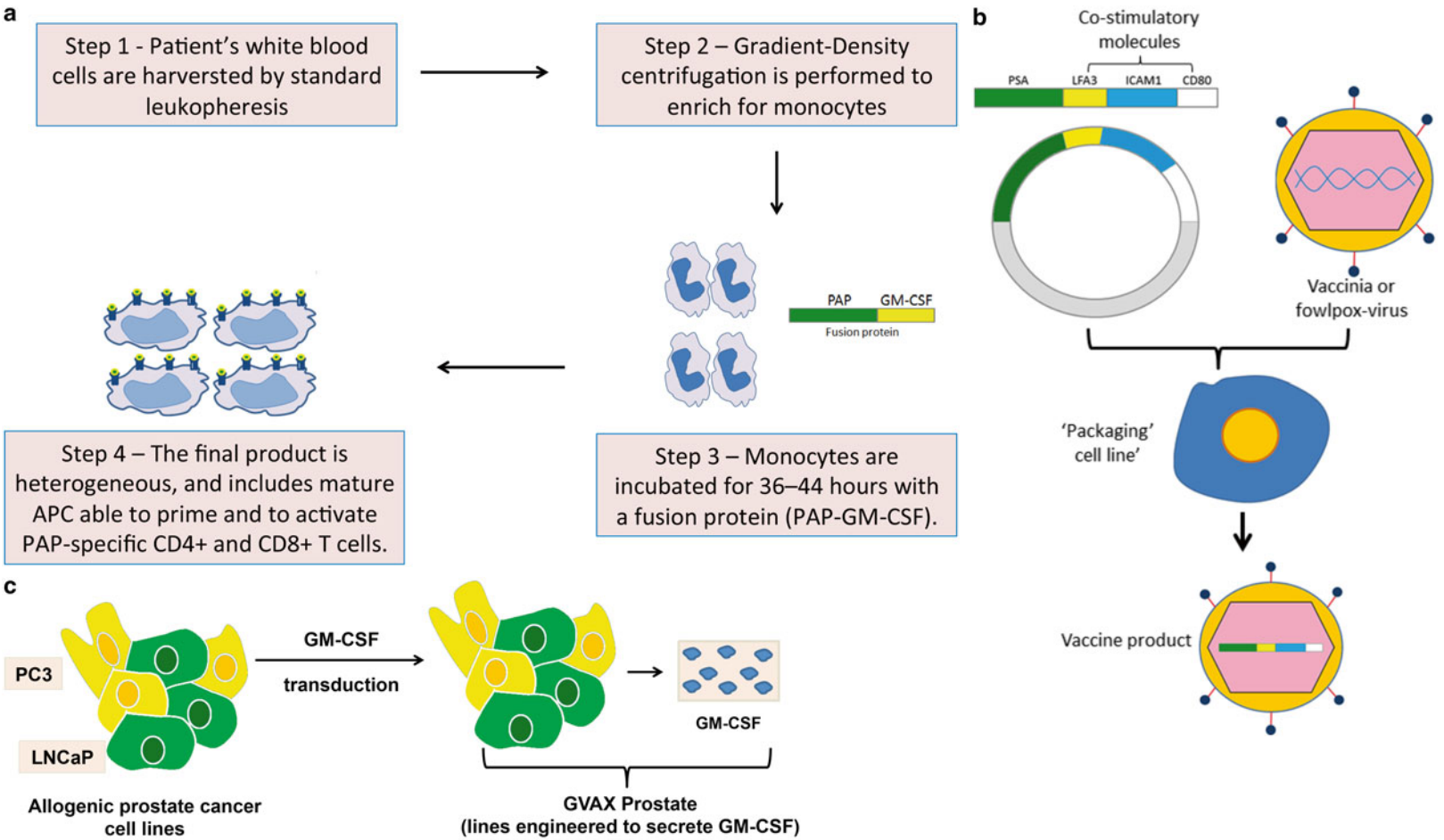

Fig. 9.1 (**a**) Sipuleucel-T: The figure outlines the steps involved in manufacturing Sipuleucel-T, an autologous vaccine product which is administered intradermally. (**b**) ProstVac VF: As detailed in the text, this is a viral-based vaccine directed against PSA. The construct includes three costimulatory molecules designed to augment an adaptive immune response. ProstVac VF is administered subcutaneously and is an off the shelf product with is not patient specific. (**c**) GVAX prostate: GVAX prostate consists of two allogeneic prostate cancer cell lines transduced to secrete GM-CSF. The vaccine product is irradiated and injected intradermally, where cells undergo necrosis and are taken up by antigen-presenting cells resident in the skin. Vaccine antigens (multiple) are taken up and presented to T cells to initiate an adaptive immune response, theoretically poly-antigenic in nature

Furthermore, these data demonstrated antigen-specific T-cell proliferation and IFN-γ ELISPOT activity in pre-culture cells obtained at weeks 2 and 4 (but not week 0). Finally, T-cell activation-associated cytokines were noted in the second and third doses of Sipuleucel-T, supporting the idea that the first infusion of activated, antigen-loaded APCs primes T cells in vivo. Thus, the second and third doses are of Sipuleucel-T are biologically different from the first, and each dose contains progressively more activated APCs and possibly a greater proportion of antigen-specific T-cells with the capacity to recognize and kill PC cells. Based on these studies, antigen-specific immune responses, in the form of either PAP-specific antibodies, or a T cell response to PAP were observed in 78.8 % of monitored subjects. Finally, the authors demonstrated a positive correlation between OS and cumulative APC activation and antigen-specific immune responses. Taken together these important analyses show that Sipuleucel-T induces an antigen-specific immune response, and that response appears to be associated with a survival benefit in treated patients.

ProstVac-VF

ProstVac VF is a poxvirus-based vaccine directed against PSA (Bavarian Nordic, USA); this technology was developed over several years by a group at the NIH [22], and its current iteration includes a number of critical modifications to optimize immunogenicity. First, the vaccine involves a heterologous prime boost in which the initial vaccine is based on a modified vaccinia ankara (MVA) backbone, followed by a series of booster vaccines with a fowlpox backbone. This is because the immune response to the MVA backbone is quite robust, so boosting with an identical vaccine is limited by the host's immune response to the viral backbone. To further increase immunogenicity, the vaccine was engineered to incorporate a triad of costimulatory molecules, including leukocyte function-associated antigen 3 (LFA-3), the T cell costimulatory molecule B7.1, and the adhesion molecule intercellular adhesion molecule 1 (ICAM-1) [23] (Fig. 9.1b). Additionally, administration of GM-CSF at the vaccination site is used to help recruit local dendritic cells (APC) and enhance antigen presentation.

Clinical Data (ProstVac-VF)

Aside from the multiple combination trials performed using ProstVac VF (see below), two randomized phase II single-agent trials in men with mCRPC provided important evidence for clinical activity. In one of these trials, 32 patients with mCRPC were enrolled, and antigen-specific immune PSA responses were shown to be associated with survival [24]. Additionally, 12 of 32 patients showed declines in serum PSA post-vaccination and 2/12 showed radiographic regression of index lesions. A second multicenter, randomized, double-blind study enrolled 125 patients randomized 2:1 to receive PROSTVAC-VF plus GM-CSF or control vectors [25]. This trial was designed with primary endpoint of time to disease progression. Although the study did not meet its primary endpoint, a significant survival advantage was observed; at 3 years post study, ProstVac-VF patients had a better OS with 25 (30 %) of 82 alive versus 7 (17 %) of 40 controls, a longer median survival (25.1 versus 16.6 months for controls, HR = 0.56, $p = 0.0061$). As in prior trials, the therapy was well tolerated and the most common AEs were injection site reactions. Systemic AEs, such as fatigue, fevers, and nausea, were reported in a subset of patients. Based on the survival benefit showed in these trials, an international randomized phase III trial has recently been initiated. This trial (NCT01322490, PROSPECT) will enroll 1,200 patients and will randomize them to either placebo, ProstVac-VF plus subcutaneous GM-CSF, or to ProstVac-VF alone. The primary endpoint of this trial is overall survival, and enrollment is limited to men with asymptomatic or minimally symptomatic mCRPC who are chemotherapy naive.

Mechanism of Action (ProstVac VF)

In vivo, poxvirus vectors most likely infect epithelial cells, a proportion of which undergo cell death. Cellular debris, including encoded antigens, are then taken up by nearby immature APCs, which, when appropriately activated, can present these antigens to CD4+ and CD8+ T cells in a pro-inflammatory context [20]. This process is known as indirect or cross-priming. Direct infection of APCs, particularly the Langerhans cells in the skin, is another mechanism by which poxvirus vectors can prime an immune response [20], and the relative role of direct versus cross-priming in patients treated with ProstVac-VF is currently not known. Nevertheless, the end result is postulated to be activation and proliferation of PSA-specific CD8 and CD4 T cells, as has been demonstrated in earlier correlative studies. Interestingly, and in contrast to the recently published data on Sipuleucel-T [21], ProstVac-VF doesn't appear to prime much of an antibody response; indeed antibodies specific for PSA have not been reported with this agent.

GVAX Prostate

GVAX prostate (Aduro Biotech, Berkeley, CA) is a cell-based immunotherapy comprised of a combination of irradiated allogeneic tumor cell lines modified with granulocyte-macrophage colony-stimulating factor (GM-CSF) gene [26] (Fig. 9.1c). The product includes two prostate carcinoma cell lines: the androgen-sensitive LNCaP, as well as the castration-resistant line PC3 [27]. The concept underlying whole-cell vaccines is that these cells will serve as a diverse source of multiple tumor and tissue specific antigens, at least some of which will correspond to antigens in the patient's tumor. These vaccines are thus considered polyvalent. The primary advantage to such an approach is that it's possible that the inclusion of multiple antigens might prevent tumors from escaping immune pressure by down-regulating the expression of a single tumor-associated antigen. The major disadvantage of GVAX vaccines is that they're relatively difficult to monitor immunologically, since the key target antigen/antigens are not known for any particular patient.

Clinical Data (GVAX Prostate)

Similar to both Sipuleucel-T and ProstVac-VF, two relatively small phase I/II trials of GVAX prostate verified safety and showed some early evidence for clinical activity. The first of these trials [28] enrolled 55 patients with chemotherapy naïve mCRPC. In men with radiographically documented metastases, the median survival in a group of men receiving a high dose of vaccine (N = 10) was 34.9 months, versus 24 months in 24 men receiving a lower dose of the vaccine. No dose limiting toxicities were noted, although injection site reactions, often prominent, were commonly observed. In a second dose-escalation trial [29], 80 men were treated, and PSA stabilization was observed in 19 % of patients, with a single patient showing a 50 % decline in PSA. Based on these trials, two multicenter randomized phase III trials were initiated. The first of these trials (VITAL-1) was a 626 patient trial in which men with asymptomatic mCRPC were randomized 1:1 to GVAX prostate (q 2 weeks × 13 doses) [30]. Interestingly, the comparator arm in this trial was not placebo, as in the ProstVac VF and Sipuleucel-T trials. Rather, standard dose docetaxel (75 mg/m^2 × 10 doses + prednisone 5 mg BID) was chosen as the comparator arm. The primary endpoint of the trial was overall survival, but the trial was halted prematurely based on an unplanned and underpowered futility analysis. Thus, it remains unknown whether GVAX prostate is capable of providing a survival advantage in men with early stage mCRPC. A second trial, Vital-2 compared the combination of GVAX prostate + chemotherapy (without prednisone) versus docetaxel chemotherapy (with prednisone) was initiated, but enrollment was halted based on a perceived imbalance in deaths, with 67 on the GVAX + chemotherapy arm, versus 47 on the chemotherapy alone arm. The trial was later closed on the basis of that "imbalance," but follow-up data showed that, in a final analysis,

there was no statistical imbalance in deaths, with 85 on the combination arm and 76 in the chemotherapy alone arm. Taken together, the two GVAX trials both support the safety of these cell-based vaccines in prostate cancer, but unfortunately no conclusions can be drawn about efficacy since both trials were halted before meeting prespecified enrollment criteria. An interesting facet of both trials is the choice of chemotherapy as a comparator arm; no other vaccine trial either before or after this has chosen chemotherapy as a comparator. At the current time, a single GVAX prostate trial is underway (NCT01696877), this is a neoadjuvant trial designed to test the ability of the vaccine to induce a T cell influx into [31] the prostate gland when administered along with hormonal therapy in the pre-surgical setting.

Mechanism of Action (GVAX Prostate)

GVAX prostate is thought to function in a manner similar to ProstVac VF—irradiated cells are administered intradermally, where they undergo necrosis and are taken up by resident dendritic cells attracted by the GM-CSF. After uptake of cellular debris and processing, antigens are presented to host CD4 and CD8 T cells in the context of host MHC molecules on the APC. This process, cross-presentation, has been demonstrated clinically [31]. As noted above, cell-based vaccines such as GVAX have the theoretical advantage of being able to present multiple tumor-related antigens simultaneously.

Prostate Cancer Vaccines in Clinical Practice

Although the focus of this chapter is the management of metastatic castration-resistant prostate cancer (mCRPC), and this is the setting where the majority of clinical trials of cancer immunotherapy have been performed, from an immunological perspective, it is important to highlight that biochemical recurrence provides a unique opportunity for immunological intervention in patients with prostate cancer. In this setting the several of the immunosuppressive mechanisms (such as Treg cells, myeloid-derived suppressor cells (MDSCs) and transforming growth factor-β (TGFβ)) associated with an advanced tumor burden are expected to be at a minimum, and it is likely that immunotherapy approaches would be more beneficial [20]. But it must be noted that the single prostate cancer vaccine that is FDA approved (Sipuleucel-T) is approved only in the metastatic state, and that clinical development of prostate cancer drugs is somewhat hampered by the absence of clear markers of response in the non-metastatic setting.

Among men with mCRPC, then, which patients are most appropriate for immunotherapy with Sipuleucel-T, and perhaps more importantly, when in the treatment sequence should this agent be given? In terms of patient status, the enrollment criteria for all 3 phase III trials of this agent stipulated that men be either asymptomatic or minimally symptomatic, so it's clear that men with advanced, symptomatic disease are most likely not appropriate candidates. In terms of sequencing, it is not well appreciated that approximately 20 % of the men treated in the phase III trial of Sipuleucel-T (IMPACT) had been treated with prior chemotherapy [19]. Subgroup analysis suggested a benefit in both chemotherapy-experienced and chemotherapy-naïve patients, so prior chemotherapy in and of itself is not an absolute contraindication to vaccine treatment, as long as patients are either asymptomatic or minimally symptomatic. Perhaps more relevant at the current time are the second-generation hormonal treatments such as abiraterone-acetate [32] and enzaluatmide [33]. These are well-tolerated agents, often administered in the pre-chemotherapy setting. Their sequencing with immunotherapy has not yet been well studied, and in the case of abiraterone acetate a particular concern arises from the notion that this agent is usually administered with a low dose of prednisone (5 mg BID). Recent data from a randomized phase II trial of Sipuleucel-T administered either concurrently (N = 30) or sequentially (N = 32) with Abiraterone acetate + prednisone (AA + P) suggested that the administration of AA + P did not appear to influence the parameters of Sipuleucel-T associated

with potency (CD54 count), and that evidence for a prime-boost effect was still documented in the product in the presence of prednisone [34]. While clinical follow-up of these patients continues, the data provide important evidence that co-administering AA + P is not obviously contraindicated. Still, though, it's not clear whether the first therapy for men with progressing mCRPC should be Sipuleucel-T or a second-generation hormonal agent. Clearly, large randomized clinical trials, following men for overall survival would be necessary to answer that question definitively. Those trials are *not* currently underway, and are in fact unlikely to be initiated. A second set of data, albeit retrospective, add a bit of additional insight. In a retrospective analysis of the large randomized phase III trial of Sipuleucel-T, patients were subgrouped based on initial PSA, and those data correlated with potential survival benefit [35]. In this study, patients with the lowest quartile of initial PSA (<22.2 ng/mL) appeared to enjoy the largest survival benefit from Sipuleucel-T (41.3 versus 28.3 months for placebo), while patients with the highest quartile of initial PSA (>134.1) appeared to achieve a less obvious survival benefit (18.4 months with Sipuleucel-T versus 15.6 months for placebo). So, taken together these data would suggest that the most appropriate patients for Sipuleucel-T treatment would be those with early stage disease, a lower PSA, and fewer symptoms.

GM-CSF as a Treatment Modality

As discussed above, GM-CSF is a component of all three vaccine approaches that have been evaluated in prostate cancer. As a cytokine, GM-CSF the most relevant role of GM-CSF is the recruitment of APC to a vaccine site, but it also has a role in APC activation. It should be noted, however, that the role of GM-CSF is not completely clear, as some studies showed that higher concentrations can lead to the induction and proliferation of a population of cells with immune suppressive properties known as Myeloid-Derived Suppressor Cells (MDSC) [36]. Whether GM-CSF induces MDSC in human cancer patients is less clear, but several studies with peptide vaccines showed that the addition of GM-CSF does not seem to significantly augment vaccine efficacy, and may in fact impair it [37]. Possibly based on those data, the phase III trial of ProstVac VF includes two vaccine arms, one with and one without GM-CSF. As an FDA-approved agent with immunological properties, GM-CSF has been tested as a single-agent in men with prostate cancer. In the first of these trials [38], GM-CSF was administered at dose of 250 mcg/m^2/day on days 1–14 of a 28-day cycle to 30 patients with biochemically recurrent prostate cancer. Approximately 10 % of the patients had a 50 % decline in PSA, and the rate of PSA rise (PSA slope) appeared to decrease in the group as a whole, suggesting an alteration in disease kinetics. Longer term follow-up of these patients showed that seven out of the initial 30 patients enrolled (24 %) remained free of disease progression a median of 5 years after starting therapy, and that those responding patients tended to have lower Gleason scores and pretreatment PSA values [39]. Despite these interesting and encouraging results, GM-CSF monotherapy in mCRPC has not been more extensively followed up in a large randomized trial setting, and the agent is not specifically FDA-approved for prostate cancer. It's worth mentioning that GM-CSF treatment has also been combined with immune checkpoint blockade using anti-CTLA-4 (see below), and here PSA responses were seen at antibody doses of 3 mg/kg and above [40].

Immune Checkpoint Blockade

As discussed above, T cells have a crucial role in anti-tumor immunity. Recent developments in basic immunology show that T cell function is physiologically modulated by the interaction of a series of cell surface proteins (called immune checkpoints) with their individual ligands [3, 41, 42]. In normal physiology, these molecules likely serve to prevent an over-exuberant immune response to infection from causing autoimmunity, but in the case of cancer these molecules likely attenuate and/or prevent an anti-tumor immune response. Perhaps the best studied of the checkpoint molecules is Cytotoxic T Lymphocyte

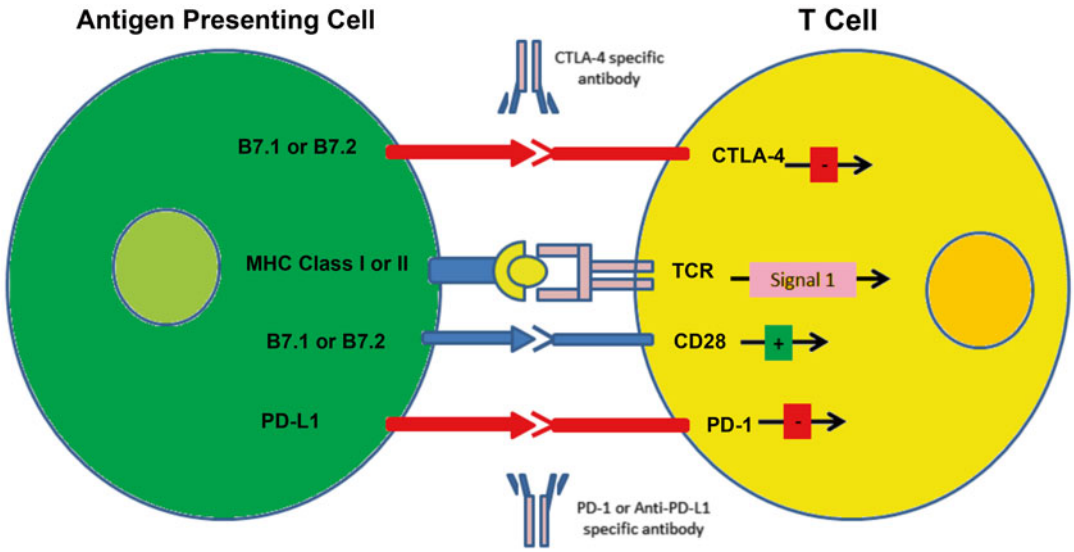

Fig. 9.2 Immune checkpoints: T cell activation requires both signal 1 (MHC + peptide/TCR) and signal 2 (B7.1 or B7.2/CD28). When a T cell up-regulates CTLA-4 (as in the tumor microenvironment), high-affinity binding of B7 molecules by CTLA-4 effectively highjacks signal 2. A monoclonal antibody directed against CTLA-4 (Ipilimumab or Tremilimumab) blocks that negative interaction, leading to T cell proliferation and effector function. The interaction between PD-L1 and PD-1 is also inhibitory, and can similarly be blocked with monoclonal antibodies directed against either PD-1 or PD-L1

Antigen-4 (CTLA-4), originally cloned as an analog of the costimulatory molecule CD28. While the function of CTLA-4 was controversial for some time [43], definitive studies using knockout mice were performed by a number of groups, with concordant results. Mice with germline deletion of CTLA-4 develop widespread lymphadenopathy and autoimmunity, and die within 21–28 days of age, attesting to a critical role for this molecule in attenuating a T-cell driven immune response [44, 45]. Subsequent studies by the Allison group showed that blocking CTLA-4 with a monoclonal antibody could potentiate anti-tumor immunity in an animal model [46]. A monoclonal anti-CTLA-4 antibody was developed clinically, and is now FDA-approved for patients with metastatic melanoma [47].

Mechanism of Action (Anti-CTLA-4)

As show in Fig. 9.2, T cell activation is a carefully orchestrated process which requires at least two distinct signals. The first signal, Signal 1, is the cognate interaction between a T cell's unique cell surface receptor (the TCR) and a surface formed by the combination of a MHC molecule and a particular peptide. The TCR signal is initiated with this is of sufficient affinity to qualify as a "good fit." But signal-1 alone is insufficient for full T cell activation, indeed T cells require a second signal, Signal 2 to acquire full effector function. Signal 2 is normally transmitted by B7 family molecules like B7.1 or B7.2 on the surface of a functional antigen-presenting cell to a receptor molecule on the T cell called CD28. In some settings, though, CTLA-4 expression is up-regulated on the surface of a T cell; this often happens in tumors or in tumor-draining lymph nodes. CTLA-4 binds to B7 molecules with higher affinity than CD28 does, effectively highjacking Signal 2 and turning that T cell off. Anti-CTLA-4 monoclonal antibodies like Ipilimumab (Bristol Myers Squibb) and Tremilimumab (Medimmune) block this interaction, essentially allowing Signal 2 to proceed and T cells to be fully activated. This strategy has proven to be effective in metastatic melanoma, and Ipilimumab is FDA-approved in that setting. As might be expected from its MOA, administration of

Ipilimumab is associated with immune-related adverse events (IRAEs), which typically include dermatitis, colitis, hepatitis, and others. IRAEs usually respond well to prompt treatment with immunosuppressive doses of corticosteroids, and treatment algorithms have been developed to assist with the management of those toxicities.

Anti-CTLA-4 (Ipilimumab) in Prostate Cancer

Ipilimumab has been evaluated in a number of early phase studies in men with prostate cancer. These data were recently summarized by Slovin et al., and show that treatment is associated with a PSA response rate of approximately 15–20 %, and with few objective (radiographic) responses [48]. In several of these studies, a low dose of radiation therapy was tested in an effort to "release antigen" and potentiate an immune response. However, in the small dataset accumulated there was no evidence for such an effect; for example, the PSA decline rate in patients treated with a dose of 10 mg/kg of Ipilimumab was 12 % in the presence of radiation therapy (RT) versus 25 % without. Despite this relatively low PSA response rate, and little evidence that low-dose RT applied to a single lesion in men with metastatic disease augmented the response rate to Ipilimumab, a phase III trial combining RT + Ipilimumab was launched in men with mCRPC who had progressed on after treatment with docetaxel chemotherapy. This trial enrolled approximately 800 men and randomized them to a single low-dose treatment with RT alone versus RT followed by Ipilimumab at a dose of 10 mg/kg q 3 weeks × 4, followed by q 3-month maintenance for men who were not progressing. The primary outcome of this trial was overall survival, and results were recently reported. Disappointingly, the trial did not meet its primary overall survival (OS) endpoint with a median OS of 11.2 months in the Ipi group versus 10.0 months for placebo, The pre-specified secondary endpoint progression free survival (PFS) was met, with PFS = 4.0 in the Ipi group versus 3.1 in the placebo group (HR = .070, $p < 0.001$). Retrospective analyses of the results showed that men with more favorable disease characteristics (no visceral metastases, normal alkaline phosphatase, normal Hgb) appeared to possibly benefit from the treatment, although that kind of a post-hoc result clearly requires verification in a prospective trial. In that line, a second trial comparing Ipilimumab versus placebo was conducted in the pre-chemotherapy setting, results from that trial are still pending at this time. Taken together, these data suggest that Ipilimumab may have some activity in mCRPC, but that patients with more favorable characteristics, particularly those without visceral metastases might be more likely to benefit.

Depletion of Regulatory T Cells

Regulatory T cells (Treg) are a population of CD4 T cells that down-modulate an immune response [49]. These cells are characterized by their expression of the transcription factor FoxP3 [50] and may either arise naturally, or be induced when a T cell encounters its cognate antigen in the presence of a suppressive environment, as in cancer. So, in cancer immunotherapy, one approach might be to deplete regulatory T cells, either alone or in combination with other therapies [51]. Interesting new data suggest that antibodies against CTLA-4 might in fact do just that, i.e. anti-CTLA-4 selectively deplete Treg based on the relatively high-level expression of CTLA-4 on tumor infiltrating Treg [52, 53]. Another way to deplete Treg in humans is the administration of low doses of the chemotherapy agent cyclophosphamide (CTX) in a prolonged or metronomic regimen [54]. Indeed, low-dose cyclophosphamide has some evidence of activity in prostate cancer [55], with recent data showing a reasonable response rate in the second line setting [56]. In neither of these studies were Treg specifically quantified, though, so the notion that Treg depletion is the major MOA for low-dose cyclophosphamide has yet to be fully explored.

Monoclonal Antibodies: Passive Immunotherapy

In the era of targeted therapy, monoclonal antibody-based treatment, which blocks proteins specifically expressed on the surfaces of tumor cells, has been established as one of the most successful therapeutic strategies for both hematologic malignancies and solid tumors in decades [57]. This approach is a passive form of immunotherapy, in that the antibodies administered are not generated in the host. In prostate cancer, prostate-specific membrane antigen (PSMA) represents a reasonable target for the development of this type of therapy [58]. PSMA is a transmembrane glycoprotein that is highly expressed in PC cells and tumor vasculature. In the normal prostate, PSMA is expressed predominantly a variant (PSM') which is restricted to the cytoplasm (Fig. 9.3). The expression of PSMA in PC cells is about tenfold greater than in normal prostate and about 50–100-fold greater than in non-prostatic tissue. Furthermore, the expression of PSMA is highly up-regulated with disease progression and is greatest in advanced disease. Early clinical trials of a fully humanized PSMA-specific monoclonal antibody (J591; Cornell Weill Medical College) showed impressive tumor targeting, but few objective clinical responses were noted in the patients with advanced tumors who were included in these studies [59]. Thus, the clinical development of J591 moved forward to a radioisotope-labeled version, with the aim of inducing cancer cell death by localizing a radioactive β emitter in close proximity to a patient's tumor mass [60]. Recently, the results of a phase II trial evaluating the radioimmunotherapy 177Lu-labeled J591 in patients with metastatic CRPC were published [61]. The radiopharmaceutical was administered in a single infusion in two different cohorts: 65 mCi/m^2 (15 patients) and 70 mCi/m^2 (32

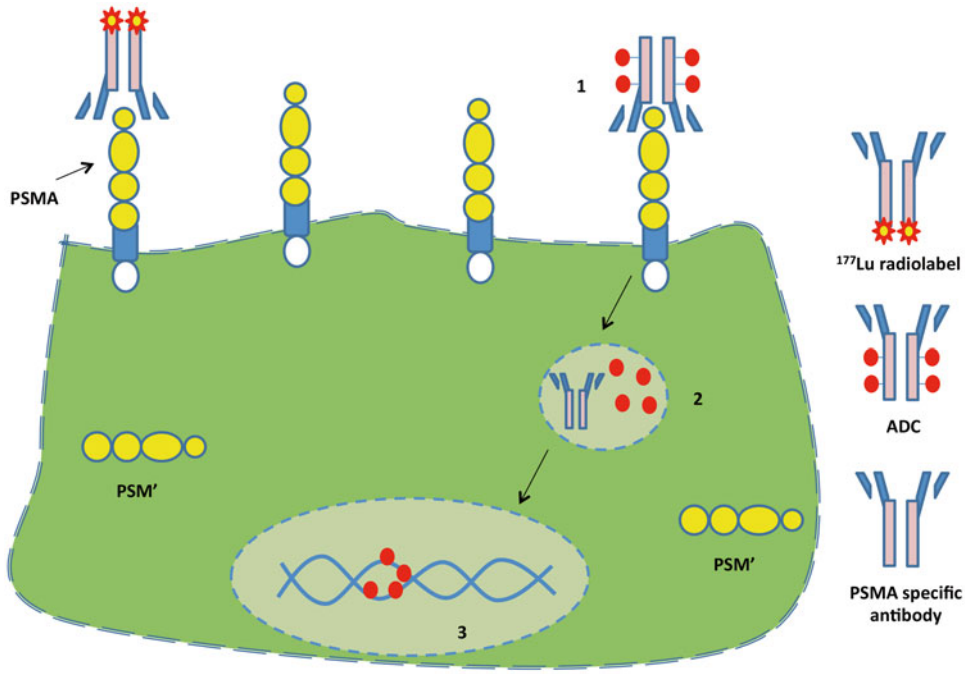

Fig. 9.3 Anti-tumor antibodies: Anti-tumor antibodies are in clinical evaluation in prostate cancer. These include "naked," i.e. unlabeled antibodies (*bottom right*), radiolabeled anti-PSMA (J591), *upper left*, and Antibody–Drug Conjugates (ADC). ADC are taken up into the cell, where the powerful chemotherapy agent dissociates from the Fc portion of the antibody, mediating tumor cell lysis

patients). The phase I maximum tolerated dose (70 mCi/m^2) resulted in more 30 % PSA declines (46.9 versus 13.3 %, $p=0.048$) and longer survival (21.8 versus 11.9 months, $p=0.03$) but also a higher rate of grade 4 neutropenia (37.5 versus 0 %, $p=0.005$) and platelet transfusions (40.6 versus 7 %). Importantly, most patients showed complete recovery of cellular levels. Currently, several trials involving 177Lu-labeled J591 are currently in progress, including studies combining this agent with conventional cancer therapy.

Another potential PSMA-based therapy is an antibody–cytotoxic drug conjugate (ADC). The PSMA ADC under current evaluation includes three components: a fully human, IgG1 anti-PSMA monoclonal antibody; a linker composed of valine-citrulline (vc) dipeptide; and the cytotoxic drug Monomethylauristatin E (MMAE), which is a synthetic dolastatin 10 analogue that potently blocks tubulin polymerization (Fig. 9.3) [62]. Like other antibody–drug conjugates, the PSMA ADC is designed to selectively bind PSMA-expressing cells, internalize via the endocytic pathway, and release MMAE. After a phase I study established a maximum tolerated dose [62], a phase II, open-label, multicenter study is ongoing to assess the anti-tumor activity and tolerability of PSMA ADC in mCRPC patients who have received at least one chemotherapy taxane-containing regimen (NCT01695044).

Combination Approaches

Multiple mechanisms contribute to the development of immune evasion as tumors evolve. Thus, it is reasonable to believe that the combination of multiple approaches, acting at different nodes in the tumor microenvironment might prove to be required to overcome suppression and promote an anti-tumor immune response. Interestingly, several common treatment modalities already widely used in prostate cancer (androgen-ablation and radiation therapy) might prove additive with immunotherapy regimens.

Androgen Ablation

During the past several years, cumulative evidence both from experimental and clinical studies demonstrates an immunosuppressive role for androgens [63]. Thus, it is of interest to note that androgen ablation, the mainstay treatment for the management of advanced prostate cancer, has key immunological effects and may combine either additively or synergistically with prostate cancer immunotherapy. Preclinical data support this rationale, showing that androgen ablation can improve vaccine efficacy [64, 65]. In addition clinical from men undergoing androgen-ablation prior to initial surgery also showed that androgen ablation results in an immunological infiltrate into the prostate gland [11, 66]. However, it is not yet clear what is the best sequence in which to administer androgen-ablation versus immunotherapy. In one way, one might consider androgen-ablation to be immunologically "priming" when administered prior to an active immunotherapy. It should also be recalled that persistent exposure to antigens renders them tolerogenic [67], so early androgen-ablation therapy might be able to boost an immune response simply by effectively eliminating that tumor burden. On the other hand, it's also possible that priming an immune response with either an active vaccine or with checkpoint blockade is an effective priming maneuver that could be boosted by androgen ablation and subsequent antigen release. To address this question in the clinic, two phase II studies were designed with immunological endpoints. The first one was randomized, phase 2 trial evaluating the optimal sequencing of Sipuleucel-T and androgen deprivation therapy (ADT) in patients with biochemically recurrent prostate cancer (BRPC). The study randomized 34 patients in each arm: Sipuleucel-T followed by ADT or ADT followed by Sipuleucel-T. Early data from this trial suggest an increase in cytokine secretion when the hormonal therapy precedes vaccination, but those data require further correlation with clinical outcomes. The second trial was a randomized, phase 2, open-label study of Sipuleucel-T with concurrent or sequential

Table 9.2 Immunological effects triggered by radiotherapy in tumor microenvironment

• Up-regulate FAS (death receptor) on tumor cell surface
• Up-regulate MHC class I on tumor cell surface
• Alter repertoire of peptides presented in class I MHC
• Translocate calreticulin to tumor cell surface, resulting in uptake by antigen-presenting cells
• Release high mobility group box 1 (HMGB1) from dying tumor cells, resulting in dendritic cell maturation

abiraterone acetate in mCRPC. As discussed above that trial showed that Sipuleucel-T could be given concurrently with abiraterone acetate + prednisone, without any appreciable decrease in product parameters [34].

Radiation Therapy (RT)

Radiation therapy has multiple immunological effects [68] (Table 9.2), including occasional descriptions of activity outside the targeted field—a so-called abscopal effect [69]. In clinical practice, the abscopal effect is rarely noted, so the frequency of such events is likely fairly low. In the laboratory, though, induction of an abscopal effect is less rare, and has been reported in several models [70, 71]. Mechanistically, this is presumed to occur because radiation, like certain chemotherapy agents, can bring about "immunogenic" cell death, in which the dying cancer cells act in a manner similar to a vaccine and prime either a new or pre-existing immune response [72]. Because the abscopal effect is thought to be immunologically mediated, there is good reason to hypothesize that immune agents like the CTLA-4 blocking antibody Ipilimumab might possibly potentiate an abscopal effect. A single case report relevant to this has been published, in which a patient who was progressing while being treated with Ipilimumab received palliative radiation therapy, and then experienced fairly widespread regression in non-targeted lesions. A plethora of trials designed to induce an abscopal effect in humans have been launched, unfortunately these trials differ drastically in the dose, schedule and fractionation of radiation therapy utilized. As mentioned above, earlier clinical experience with Ipilimumab in prostate cancer doesn't support the notion that a low dose (8 Gy) of radiation to a single lesion is insufficient to induce an abscopal effect in the setting of widespread metastatic disease [48]. However, more robustly eliminating the tolerogenic tumor burden by treating men with oligometastatic disease, or earlier in the disease course has yet to be tested formally.

Chemotherapy

Chemotherapy is challenging to integrate with immunotherapy. This is because chemotherapy administered at the standard near-maximum tolerated doses is generally immunosuppressive, and because only certain types of chemotherapies (for unclear reasons) are capable of augmenting rather than tempering an anti-tumor immune response. Of the many chemotherapy regimens possible, we found that single-agent cyclophosphamide could augment the immune response to a vaccine in a genetically engineered model of prostate cancer [73]. However, this was only accomplished when cyclophosphamide was administered at a low dose (approximately 1/3 of the normal dose) and in a specific sequence (1 day prior to immunotherapy). Other regimens led to a diminution of the immune response. These results have been mirrored in several human clinical trials, perhaps most elegantly in a study in breast cancer wherein the Emens group found that either low-dose cyclophosphamide given 1 day prior to vaccination, or low-dose Adriamycin given 1 day post-vaccination augmented an anti-tumor immune response [74]. In terms of prostate cancer, we have extensively explored the combination of docetaxel with vaccination in a genetically engineered preclinical model, but were unable to find any regimen that did not lead to a decreased immune response (C. Drake, unpublished). As mentioned above, cyclophosphamide does show considerable promise in this regard, and it's fairly benign safety profile increases enthusiasm for combination studies [75].

Conclusions

Prostate cancer is unique in that it was the first solid tumor in which an active vaccine (Sipuleucel-T) was approved for treatment for metastatic patients [19]. This sparked renewed interest in vaccine therapies, and the poxvirus-based product known as ProstVac VF is now in a large phase III study. Immune checkpoint blockade, while showing considerable promise in kidney cancer, lung cancer, and melanoma [76] has not been especially active in prostate cancer [77], although a trial of the CTLA-4 blocking antibody in earlier stage disease is currently in progress. Moving forward, it is likely that combining immunotherapy with either androgen-ablation or radiation therapy, or perhaps combining multiple immunotherapies [78, 79], may be required for ultimate success.

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Bone-Targeted Therapy: Rationale and Current Status

10

Benjamin A. Gartrell and Fred Saad

Prostate Cancer and Complications in Bone

Prostate cancer will be diagnosed in approximately 238,590 men in the USA in 2013 and roughly 29,720 will die of the disease [1]. Worldwide it is estimated that 903,500 cases are diagnosed with 258,400 deaths annually [2]. Bone involvement is present in approximately 90 % of patients with metastatic disease [3]. Bone metastases are commonly associated with skeletal-related events (SREs), which are generally defined as bone metastases leading to pathologic fracture, spinal cord compression or the requirement of radiotherapy or orthopedic surgery to bone. The use of androgen deprivation therapy (ADT) in the treatment of advanced prostate cancer patients without bone metastases results in loss of bone mineral density (BMD) with a subsequent increase fracture risk [4]. However, the risk of fragility fracture in men is influenced by multiple risk factors.

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Bone Physiology

Normal healthy bone requires a coordinated interaction between osteoblasts and osteoclasts. Osteoblasts form new bone while osteoclasts resorb bone resulting in a dynamic steady state required for maintenance of normal bone integrity. Osteoblasts are of mesenchymal origin and secrete non-calcified osteoid, which is composed largely of type 1 collagen. Osteoblasts are also responsible for mineralization of osteoid. Osteoclasts are derived from monocyte/macrophage precursor cells and secrete factors lytic enzymes which degrade mineralized bone matrix [5]. The relative function of osteoblasts and osteoclasts is critical in maintaining appropriate levels of BMD and the molecular mechanisms at play have been elucidated. Thus, it is now clear that bone is dynamic and an intricate communication between cellular elements within the bone influenced by both local and systemic factors controls a constant process of bone renewal.

Several members of tumor necrosis factor (TNF) and TNF receptor (TNFR) superfamily control osteoclast differentiation and function. Osteoprotegerin (OPG) is a secreted member of the TNFR superfamily which was found to prevent osteoclast differentiation in vitro and to cause osteopetrosis when overexpressed in animal models [6, 7]. While other members of the TNFR superfamily are transmembrane proteins, OPG lacks a transmembrane domain. Thus, it

F. Saad and M.A. Eisenberger (eds.), *Management of Castration Resistant Prostate Cancer*, Current Clinical Urology, DOI 10.1007/978-1-4939-1176-9_10,

was suspected that this secreted, soluble protein acted to bind a ligand thereby preventing the ligand from interacting with a cellular target [6, 7]. Subsequent studies identified receptor activator for nuclear factor κB ligand (RANKL) as the TGF family cytokine which OPG bound [8, 9]. In the presence of colony-stimulating factor-1 (CSF-1), RANKL was shown to stimulate osteoclastogenesis and to promote osteoclast function and survival [8, 9]. RANKL is a transmembrane protein that may undergo proteolytic cleavage to a soluble form (sRANKL) [8]. Sources of RANKL include osteoblasts, stromal cells, and T-cells [9, 10]. RANK–RANKL signaling is also important for T-cell and dendritic cell function [11–13]. RANK is expressed by both osteoclast precursors and mature osteoclasts and transgenic knockout mice have severe osteopetrosis as osteoclastogenesis and function is severely impaired [14, 15]. RANK–RANKL signaling also plays a key role in maintaining calcium metabolism.

Bone Pathophysiology in Advanced Disease

ADT results in profound decrease in serum testosterone and estradiol and these hormone changes result in loss of BMD and increase in fracture risk [16, 17]. The estrogen receptor is expressed by both osteoclasts and osteoblasts [18]. The primary estrogen in men is estradiol, which is produced from testosterone and androstenedione by aromatase in multiple tissues. The role of estrogen in female bone health has been well established, but men with mutations in the estrogen receptor have decreased BMD and incomplete epiphyseal closure [19]. Thus, the primary mediator of decreased BMD and increased fracture risk in men appears to be a decrease in estradiol.

On radiography bone metastases in prostate cancer are generally osteoblastic. However, both osteoblast and osteoclast activity are upregulated in the presence of bone metastases from prostate cancer [18]. While other cancers are characterized by bone metastases, prostate cancer is unique in that bone is often the only site of metastatic disease. The reasons for tropism of metastatic prostate cancer to bone are not entirely clear, but an interaction between chemokine receptor 4 (CXCR4), which is expressed on prostate cancer cells and its receptor, stromal-derived factor-1 (SDF-1) which is expressed on bone stromal cells, appears to be of importance [20]. Androgens induce expression of CXCR4 by interaction of the androgen-regulated transcription factor ERG with the CXCR4 promoter [21]. Signaling mediated by the SDF-1/CXCR4 signaling pathway has been shown to regulate androgen receptor nuclear localization in prostate cancer cells [22]. Thus, androgens may contribute to the tropism of prostate cancer to bone. Integrins such as αVβ3 are expressed on prostate cancer cells and target prostate cancer cells to molecules expressed in the bone microenvironment such as osteopontin and vitronectin [23]. Activation of CD44 and αVβ3 pathways leads to phosphorylation and nuclear localization of the transcription factor RUNX2 the targets of which include multiple matrix metalloproteinases (MMPs) and RANKL [24]. Thus, integrins may participate in the homing of prostate cancer cells to bone and then participate in the pathophysiology after arrival in the bone microenvironment.

The interaction of prostate cancer cells with the bone microenvironment leads to signaling which both drives cancer progression and potentiates destruction of bone. This process has been termed the "vicious cycle" [25] (Fig. 10.1). According to this model, products of osteolysis serve as growth factors for cancer cells, which generate products that drive osteoblast and stromal production of osteoclastic factors. These osteoclastic factors drive osteoclast mediated resorption of bone with release of additional factors from matrix further perpetuating the "vicious cycle." Several molecular drivers of this process warrant particular attention.

Transforming growth factor beta (TGFβ) is released from resorbed bone matrix and has been shown to augment signaling through parathyroid hormone-related protein (PTHrP) [26]. Prostate cancer cells metastatic to bone express PTHrP [27].

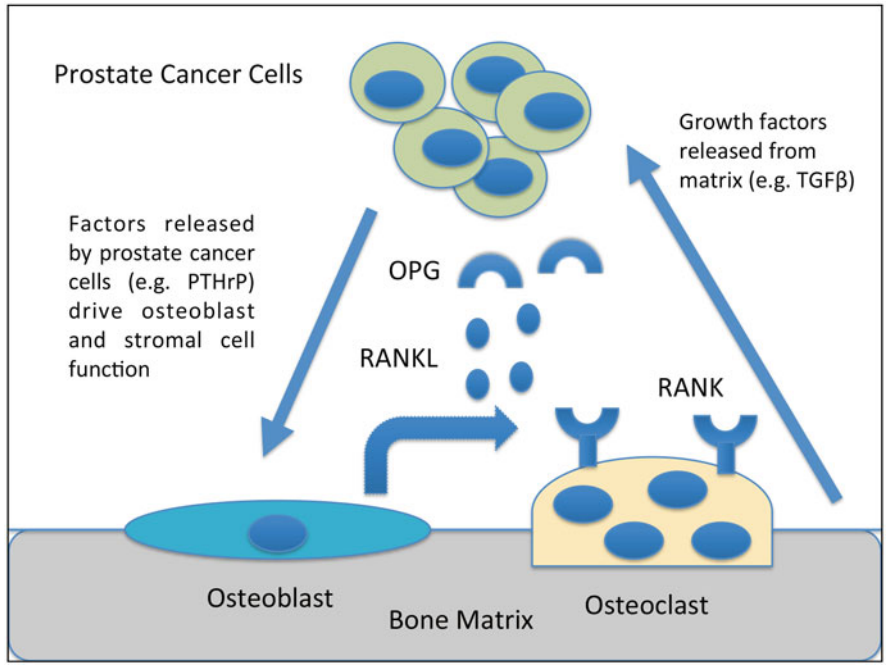

Fig. 10.1 The vicious cycle of bone metastases

Parathyroid hormone receptor is expressed by osteoblasts and stromal cells and signaling through this receptor leads to upregulation in RANKL [28, 29]. NF-κB upregulates expression of PTHrP and RANKL by prostate cancer cells [30]. Calcium is released from resorbed bone. Prostate cancer cells express the calcium-sensing receptor (CaSR) and calcium has been shown to induce proliferation of prostate cancer cells [31]. Thus, TGFβ and calcium are among the growth factors released from bone via osteolysis which fuel cancer cell release of PTHrP, which mediates generation of osteoclastic factors thereby driving the "vicious cycle."

The bone matrix contains many additional growth factors including fibroblast growth factor (FGF), platelet-derived growth factor (PDGF), and Insulin-like growth factor 1 (IGF1) [39]. These and other growth factors likely contribute to prostate cancer growth in the bone microenvironment [40]. Many other molecules have been implicated in the "vicious cycle." These include endothelin 1 [32], the bone morphogenic proteins (BMPs) [33], Wnt signaling and an inhibitor of Wnt signaling, DKK-1 [34, 35], hypoxia-inducible factor 1 (HIF1) [36], tumor necrosis factor alpha (TNFα) [37], and urokinase-type plasminogen activator (uPA) [38].

SREs in Advanced Disease

As previously stated, bone metastases in advanced prostate cancer are very common. As one might expect, disease metastatic to bone is frequently associated with SREs. Approximately one-half of patients with mCRPC to bone will experience an SRE in a 2-year period in the absence of bone-targeted therapy and 33 % will require radiation to bone, 25 % will experience a pathologic fracture and 8 % will have spinal cord compression [39]. The consequences of SREs to patients are significant. The health-related quality of life (HRQOL) as determined by the Functional Assessment of Cancer Therapy-General (FACT-G) scale shows a decrease in total score and a decrease in subscale scores measuring physical, emotional, and functional well-being in patients with prostate

cancer having experienced an SRE compared to patients not having experienced an SRE [40]. Patients having experienced an SRE also report worse pain as determined by scores of the Brief Pain Inventory (BPI) [40]. Perhaps most significantly, survival is worse in prostate cancer patients having experienced an SRE [40, 41].

Bone markers have been identified that allow the rate of bone turnover to be measured. Markers such as bone-specific alkaline phosphatase and osteocalcin are markers of bone formation. Markers of bone resorption include calcium and hydroxyproline. These markers lack specificity and therefore utility. Bone turnover markers specific to bone resorption include the degradation products of type 1 collagen such as N-terminal telopeptide (NTx), C-terminal telopeptide (CTx), pyridinium cross-links pyridinoline (PYD), and deoxypyridinoline (DPD). Elevated levels of bone turnover markers are associated with progression of cancer, increased SRE risk and decreased survival [42].

Fragility Fractures Associated with ADT

While ADT is used in metastatic prostate cancer, it is also commonly used in men without metastases. ADT results in profound hypogonadism, which is a major risk factor for osteoporosis in men [43]. Initiation of ADT in men with non-metastatic prostate cancer is associated with loss of BMD and an increased risk of fragility fractures [44, 45]. Multiple clinical risk factors help to predict fracture risk including age, personal history of fragility fracture, family history of fragility fracture, smoking, excessive alcohol consumption, and corticosteroid use [46]. The fracture risk assessment tool (FRAX) is used to predict fracture risk by integrating clinical risk factors with or without BMD [47].

Bisphosphonates in Advanced Prostate Cancer

Bisphosphonates are structural analogues of pyrophosphate and are adsorbed onto hydroxyapatite in the extracellular matrix of bone where they inhibit osteoclast mediated bone resorption. First-generation bisphosphonates such as clodronate and etidronate are relatively low-potency antiresorptive agents. Second-generation molecules such as pamidronate and alendronate have a nitrogen-containing side chain that confers increased potency. Third-generation molecules such as ibandronate, risedronate, and zoledronic acid are the most potent antiresorptive bisphosphonates.

Clodronate was evaluated in a placebo-controlled, randomized trial in 311 patients with castrate-sensitive prostate cancer with bone metastases. Clodronate failed to achieve a statistically significant improvement in bone progression-free survival or overall survival [48]. Pamidronate has been studied in placebo-controlled trials in subjects with mCRPC. In two phase II randomized studies including 378 patients, pamidronate did not reduce the incident of SREs [49].

Zoledronic acid was evaluated in a placebo-controlled phase III trial in patients with mCRPC metastatic to bone [50] (Table 10.1). In the 039 trial, 643 patients were randomized to zoledronic acid or placebo IV every 3 weeks. The trial initially included cohorts of 4 and 8 mg of zoledronic acid. However, given evidence of renal toxicity, the 8 mg cohort was reassigned to 4 mg and infusion time was increased from 5 to 15 min. Treatment with zoledronic acid lead to a statistically significant decrease in the incidence of SREs at 15 months (33.2 vs. 44.2 %, $p=0.021$). The time to first SRE was 488 days for the 4 mg zoledronic acid group and 321 days for the placebo group ($p=0.009$) [39]. A modest effect on pain was noted with a statistically significant advantage in pain for the 8/4 mg cohort versus placebo.

Adverse events more common with zoledronic acid included fatigue, anemia, fever, myalgia, and lower extremity edema. Severe grade hypocalcemia was uncommon with zoledronic acid (2 %). After the trial amendment, rates of renal deterioration were similar with zoledronic acid compared to placebo (15.2 vs. 11.5 %). Though ONJ was not reported, an association between bisphosphonate use and ONJ was not reported until after publication of the study results [51].

Table 10.1 Key trials of zoledronic acid and denosumab in advanced prostate cancer

Study	N	Population	Arm 1	Arm 2	Treatment duration (months)	Primary outcome	Regulatory approval
Zoledronic acid 039 [50]	643	mCRPC	Zoledronic acid IV Q3 weeks	Placebo	15[a]	Proportion of subjects with an SRE at 15 months 33.2 % (arm 1) vs. 44.2 % ($p=0.021$)	Yes
Denosumab HALT 138 [45]	1,468	Non-metastatic prostate cancer on ADT	Denosumab 60 mg SC Q6 months	Placebo	36	BMD at LS at 24 months +5.6 (arm 1) vs. −1.0 % ($p<0.001$)	Yes
Denosumab 147 [55]	1,432	Non-metastatic CRPC	Denosumab 120 mg SC Q4 weeks	Placebo	20.2 (arm 1) 19.0 (arm 2)	Bone-metastasis-free survival 29.5 (arm 1) vs. 25.2 months ($p=0.028$)	No
Denosumab 103 [56]	1,904	mCRPC	Denosumab 120 mg SC + Placebo IV Q4 weeks	Zoledronic acid 4 mg IV + Placebo SC Q4 weeks	12.2 (arm 1) 11.2 (arm 2)	Time to first on-study SRE 20.7 (arm 1) vs. 17.1 months ($p=0.0002$)[b]	Yes

[a]This was a fixed-interval study. A proportion of patients ($n=122$) continued treatment for a total of 24 months
[b]For non-inferiority

Zoledronic acid has also been evaluated in two randomized studies to evaluate the prevention of bone metastases. One trial in men with non-metastatic CRPC and rising PSA with no bone metastases was closed prematurely to accrual after an interim analysis revealed a lower than expected event rate [52]. The ZEUS trial included 1,433 subjects with high-risk prostate cancer that were randomized to zoledronic acid every 3 months for 4 years or observation. Results of ZEUS were presented at the European Association of Urology 28th Annual Congress in 2013. There was no difference in rates of bone metastases between the two groups.

Bisphosphonates have been shown to improve BMD relative to controls in patients with non-metastatic prostate cancer treated with ADT [53, 54]. However, no trial of a bisphosphonate in this setting has been powered to detect a difference in fracture risk.

Zoledronic acid has received regulatory approval for use in prostate cancer. In the USA, approval stipulates that prostate cancer patients with bone metastases must have failed at least one hormonal agent. Both zoledronic acid and pamidronate have received regulatory approval for the treatment of hypercalcemia of malignancy.

Denosumab in Advanced Prostate Cancer

Denosumab is a monoclonal antibody targeting RANKL. As previously stated, RANK–RANKL signaling promotes osteoclastogenesis as well as osteoclast survival and function. Denosumab has been evaluated in multiple settings in advanced prostate cancer and these studies will be reviewed here.

In the Hormone Ablation Bone Loss Trial (HALT 138), 1,468 men with non-metastatic prostate cancer treated with ADT were randomized to receive denosumab 60 mg or placebo SC every 6 months [45] (Table 10.1). This trial included patients at high-risk for fragility fractures based on the following criteria; age ≥ 70, decreased BMD, or a history of a fragility fracture. Patients were stratified by age (<70 or ≥70) and duration of ADT (≤6 or >6 months). The primary outcome measure was BMD at the lumbar spine at 24 months. Secondary endpoints included the incidence of new vertebral fractures at 36 months, fracture at any site, and time to first clinical fracture.

BMD in the lumbar spine increased by 5.6 % at 24 months with denosumab and decreased by 1.0 % with placebo ($p<0.001$). New vertebral fractures were more common with placebo (3.9 vs. 1.5 %; $p=0.006$). The incidence of any fracture favored denosumab but did not reach statistical significance (7.2 vs. 5.2 %; $p=0.10$). Fractures occurring at more than one site were more common with placebo (2.5 vs. 0.7 %; $p=0.006$). There was no difference in time to first clinical fracture between the two groups. Bone turnover markers decreased with denosumab relative to placebo. Cataracts were more common with denosumab. No additional adverse events were clearly more common with denosumab. An increased incidence of cataracts has not been seen in any other studies of denosumab. Denosumab has received regulatory approval for use in men at high-risk for fracture with non-metastatic prostate cancer treated with ADT.

Denosumab has been evaluated to delay bone metastases in patients with non-metastatic CRPC [55] (Table 10.1). This trial included 1,432 patients at high-risk for developing metastatic disease as defined by a PSA of ≥8.0 μg/L or a PSA doubling time of ≤10 months. Subjects were stratified by PSA criteria (one or both) and by previous chemotherapy (yes or no). Participants were randomized to denosumab 120 mg or placebo SC every 4 weeks. Bone scans were performed every 4 months and skeletal surveys were done on an annual basis. The primary efficacy measure was bone-metastasis-free survival and favored denosumab (29.5 vs. 25.2 months; $p=0.028$). Denosumab treatment also significantly delayed the time to first bone metastasis and decreased the incidence of symptomatic bone metastases. However, there was no improvement in progression-free or overall survival with denosumab.

The incidence of ONJ was 5 % with denosumab. Severe grade hypocalcemia occurred in

1.3 % of patients treated with denosumab. Based on the lack of survival difference and the high incidence of adverse events, most notably ONJ, the United States Food and Drug Administration rejected approval of denosumab to delay bone metastases in patients with non-metastatic CRPC.

Zoledronic Acid Versus Denosumab in mCRPC

Zoledronic acid has been compared to denosumab for the prevention of SREs in men with CRPC and bone metastases [56] (Table 10.1). In this randomized, double-blinded study, 1,904 subjects were randomized to denosumab 120 mg SC and placebo IV every 4 weeks or to zoledronic acid at 4 mg IV and placebo SC every 4 weeks. Stratification factors included previous SRE (yes or no), PSA (<10 or ≥10 mg/ml), and chemotherapy for prostate cancer in 6 weeks prior to randomization (yes or no). The primary efficacy endpoint was time to first on-study SRE and favored denosumab (20.7 vs. 17.1; $p=0.0002$ for non-inferiority and $p=0.008$ for superiority). Denosumab was a more potent suppressor of serum bone-specific alkaline phosphatase and of uNTx.

Denosumab caused more hypocalcemia (13 vs. 6 %; $p<0.0001$). The incidence of ONJ was 2.3 % with denosumab and 1.3 % with zoledronic acid ($p=0.09$). No increased incidence of adverse events potentially related to renal impairment was seen with zoledronic acid. Acute phase reactions were more common with zoledronic acid. Denosumab has been approved for use in solid tumors with bone metastases. Unlike the approval of zoledronic acid, approval for denosumab in this population does not stipulate castration-resistance.

Radiopharmaceuticals

Radiopharmaceuticals have several potential advantages relative to the use of external beam radiotherapy in the treatment of bone metastases. These agents are deposited in bone and preferentially at sites of active bone turnover such as areas of bone metastases with a relative sparing of uninvolved bone marrow [57]. As such, all bone metastases in an individual patient are exposed to these agents with minimal exposure to uninvolved tissues.

Beta-emitting radiopharmaceuticals include strontium-89 and samarium-153 conjugated to lexidronam (EDTMP). While beta-emitters have been shown to palliate pain, no survival advantage has been demonstrated [58]. The range of beta particles is on the order of millimeters [59], which suggests that bone marrow elements adjacent to metastases are likely to receive some radiation. Thus, despite the targeting of these radiopharmaceuticals to sites of bone metastases, the major toxicity is myelosuppression. Strontium-89 is associated with an approximately 20–30 % decrease in platelet and leukocyte count with a nadir approximately 6 weeks following treatment, with severe grade hematologic adverse events being uncommon in appropriately selected patients [57, 60]. Samarium is associated with a similar degree of myelosuppression [61, 62].

More recently, the alpha-emitting radiopharmaceutical radium-223 dichloride (alpharadin) has been developed for use in mCRPC. Compared to beta-emitters, alpha-emitting radioisotopes have a shorter range of tissue penetration of <100 μm. This corresponds to only several cell diameters resulting in less exposure to uninvolved bone marrow. Alpha-emitters also have a higher linear-energy transfer conferring them with a greater ability to cause DNA damage in prostate cancer cells. DNA and thus kill cancer cells.

In the Alpharadin in Symptomatic Prostate Cancer Patients (ALSYMPCA) study 921 patients with CRPC and bone metastases were randomized 2:1 to receive six monthly injections of intravenous radium-223 or placebo [63]. Subjects were required to have two or more bone metastases. Subjects were required to have symptoms attributable to bone metastases as determined by the regular use of analgesics or treatment with external radiotherapy for bone metastases in 12 weeks prior to randomization. Participants were not required to have received previous chemotherapy. Stratification factors included previous treatment with docetaxel (yes or no), alkaline phosphatase level (<220 or ≥220 units/L), and current bisphosphonate use

Table 10.2 Impact of disease modifying agents on skeletal morbidity

Study	N	Population	Agent	Overall survival	SREs
COU-AA-301 [64]	1,195	mCRPC (prior chemo)	Abiraterone with prednisone	14.8 vs. 10.9 months; HR, 0.65; $p<0.001$	9.9 vs. 4.9 months[a]
AFFIRM [65]	1,199	mCRPC (prior chemo)	Enzalutamide	18.4 vs. 13.6 months; HR, 0.63; $p<0.001$	16.7 vs. 13.3 months; HR, 0.69; $p<0.001$[b]
ALSYMPCA [63]	921	mCRPC (with bone pain)	Radium-223	14.9 vs. 11.3 months; HR, 0.70; $p<0.001$	15.6 vs. 9.8 months; HR, 0.66; $p<0.001$[c]

[a]Time until 25 % of cohort with a skeletal event. This was an exploratory endpoint
[b]Time to first on-study SRE. This was a secondary endpoint
[c]Time to the first symptomatic skeletal event. This was a secondary endpoint

(yes or no). Exclusion criteria included a history of visceral metastatic disease or lymph node metastases of greater than 3 cm in greatest dimension. The primary outcome measure was overall survival. Secondary endpoints included the time to first symptomatic skeletal event. Median overall survival was superior in subjects treated with radium-223 [14.9 vs. 11.3 months; hazard ratio (HR), 0.70; 95 % confidence interval (CI) 0.58–0.83; $p<0.001$]. The median time to first symptomatic SRE was also longer with radium-223 (15.6 vs. 9.8 months; HR, 0.66; 95 % CI 0.52–0.83; $p<0.001$) (Table 10.2). Other secondary endpoints such as PSA and alkaline phosphatase response also favored radium-223. Radium-223 was well tolerated with no clear increase in severe grade hematologic or non-hematologic adverse events relative to placebo. Radium-223 has received regulatory approval for use in CRPC with symptomatic bone metastases and no visceral metastatic disease.

Other Agents that Reduce SREs

A number of recently approved agents in the treatment of mCRPC have been demonstrated to delay SREs. In the COU-AA-301 trial, 1,195 patients with mCRPC previously treated with chemotherapy were randomized 2:1 to treatment with abiraterone plus prednisone or placebo plus prednisone [64]. Overall survival, the primary endpoint, favored abiraterone (14.8 vs.10.9 months; $p<0.001$). An exploratory endpoint, the time at which 25 % of participants experienced a skeletal event favored the abiraterone group (9.9 vs. 4.9 months) (Table 10.2).

In the AFFIRM trial, enzalutamide was evaluated in patients with mCRPC who had received previous chemotherapy [65]. In this trial, 1,199 participants were randomized 2:1 to enzalutamide or placebo. Median overall survival was longer with enzalutamide (18.4 vs. 13.6 months; $p<0.001$). The time to first SRE was evaluated as a secondary endpoint and favored enzalutamide (16.7 vs. 13.3 months; HR, 0.69; $p<0.001$) (Table 10.2).

Disease progression is a risk factor for the development of SREs [66]. Therefore, it is not surprising that active agents that have a survival advantage are also associated with a delay in SREs. This has invited some to question if osteoclast inhibitors are as important in an era with better treatment options for mCRPC. In a post hoc analysis of data from the COU-AA-302 trial evaluating abiraterone in patients with mCRPC who were asymptomatic or mildly symptomatic and had not previously been treated with chemotherapy, the impact of treatment with a concomitant bone-targeted therapy was evaluated [67]. Bone-targeted therapy was associated with a delay in symptomatic progression, delay in

decline of functional status and improved survival suggesting that bone-targeted agents provide additional benefit to abiraterone in this setting. In the TRAPEZE trial, subjects with mCRPC were treated with docetaxel and were randomized to receive zoledronic acid, strontium-89, or both agents. Zoledronic acid was associated with an improved SRE free interval (18.1 vs. 13.1 months; $p=0.008$) [68]. These trials demonstrate that bone-targeted agents remain indispensable in appropriately selected patients with mCRPC.

Potential Complications of Bone-Targeted Agents

Osteonecrosis of the Jaw

Osteoclast inhibitors are associated with ONJ (Table 10.3). ONJ refers to the presence of exposed bone in the jaw that persists for at least 2 months despite appropriate therapy [69]. The first case reports of ONJ associated with bisphosphonate use surfaced in 2003 [51]. The incidence of ONJ varies between osteoclast inhibitors and with dosing of these agents. Higher rates of ONJ appear to occur with more potent osteoclast inhibitors and with higher or more frequent dosing of these drugs. In the placebo-controlled trial of zoledronic acid in mCRPC with bone metastases, no cases of ONJ were reported, but an association with these agents and ONJ had not yet been established at the time this trial was reported [50]. No cases of ONJ were reported in the trial of denosumab given at 60 mg every 6 months in patients with prostate cancer on ADT [45]. In the bone metastasis prevention trial, ONJ was reported in 5 % of patients given denosumab at 120 mg monthly [55]. In the trial comparing denosumab to zoledronic acid in mCRPC, ONJ occurred in 2.3 and 1.3 % of subjects, respectively ($p=0.09$) [56].

A combined analysis of the three randomized trials comparing denosumab and zoledronic acid evaluated the incidence, risk factors, and outcomes of ONJ in these patients who had a variety of solid tumors and multiple myeloma [70]. The analysis included data from 5,723 patients. The incidence of ONJ with denosumab was 1.8 versus 1.3 % with zoledronic acid. This difference did not reach statistical significance.

Table 10.3 Notable adverse events reported in key trials of zoledronic acid and denosumab

Study	Treatment arms	Grade ≥3 AE (%)	ONJ (%)	Renal (%)	Hypocalcemia (%)
Zoledronic acid 039 [50]	(1) Z 4 mg Q3wk (2) Z 4/8 mg Q3wk (3) P	NR	NR	Renal deterioration (1) 21 (2) 15 (3) 13	Grade 3–4 (1) 1.9 (2) 2.0 (3) 0
Denosumab HALT 138 [45]	(1) D 60 mg Q6 months (2) P	(1) 35 (2) 31	(1) 0 (2) 0	NA	Any grade (1) 0.1 (2) 0
Denosumab 147 [55]	(1) D 120 mg Q4wk monthly (2) P	(1) 46 (2) 46	(1) 5 (2) 0	NA	Any grade (1) 1.7 (2) 0.3
Denosumab 103 [56]	(1) D 120 mg Q4 weeks (2) Z 4 mg Q4 weeks	(1) 63 (2) 60	(1) 2.3 (2) 1.3	AEs potentially related to renal dysfunction (1) 15 (2) 16	Any grade (1) 13 (2) 6

Z Zoledronic acid; *P* Placebo; *D* Denosumab; *NR* Not requested; *NA* Not applicable; *ONJ* Osteonecrosis of the jaw; *AE* Adverse events

The majority of patients that developed ONJ required only conservative measures (54 %) or limited surgical debridement (41 %). Surgical resection of bone was required in less than 5 % of patients diagnosed with ONJ. The primary risk factor for the development of ONJ was a history of dental extractions. Dental extraction as a risk factor for ONJ has been reported in multiple studies [71–73]. A preventative approach with dental evaluation prior to initiation of osteoclast inhibitors may reduce the incidence of ONJ [74, 75]. In addition, if invasive dental work is required in a patient receiving an osteoclast inhibitor, these agents should be held prior to the dental procedure and only restarted after complete healing has occurred.

Hypocalcemia

Hypocalcemia is an adverse event seen with osteoclast inhibitors (Table 10.3). In the 039 trial of zoledronic acid to prevent SREs, severe grade hypocalcemia was seen in 1.9 % of subjects. With low dose denosumab (60 mg every 6 months) to improve bone health in men with prostate cancer on ADT, severe grade hypocalcemia is very uncommon. In the phase III trial comparing denosumab (120 mg monthly) versus monthly zoledronic acid to prevent SREs, hypocalcemia was more common in participants treated with denosumab (13 vs. 6 %, $p<0.0001$). Severe grade hypocalcemia was also more common with denosumab (5 vs. 1 %). In most cases, hypocalcemia related to treatment with osteoclast inhibitors has been asymptomatic, but fatal events attributed to hypocalcemia have been reported.

Of special note, denosumab at 120 mg has not been evaluated in patients with severe renal dysfunction (GFR ≤ 30 ml/min). Though denosumab is not nephrotoxic and is not cleared by the kidney, there appears to be a greater likelihood of hypocalcemia in patients with severe renal dysfunction or those on dialysis treated with denosumab. In a report of 55 patients with various degrees of renal dysfunction treated with a single dose of denosumab at 60 mg, the risk of hypocalcemia correlated with degree of renal impairment [76]. However, it should be noted that even in this report, following initiation of adequate supplementation with calcium and vitamin D, no further cases of hypocalcemia occurred.

All patients treated with denosumab or zoledronic acid should have normal calcium levels prior to initiation of therapy, should be given supplemental calcium and vitamin D (unless there is a contraindication such as pre-existing hypercalcemia) and all patients should have regular monitoring of calcium levels. Special consideration should be given to patients with severe renal dysfunction. In such patients denosumab use is likely associated with a greater incidence of hypocalcemia.

Renal Insufficiency

Bisphosphonates are potentially nephrotoxic whereas denosumab has no impact on renal function (Table 10.3). In the 039 trial, after an amendment to eliminate the 8 mg dose of zoledronic acid and to administer the drug over a longer period of time, rates of renal deterioration were only mildly higher in patients treated with zoledronic acid (15.2 %) compared to those receiving placebo (11.5 %). In the 103 trial of denosumab versus zoledronic acid adverse events potentially related to renal insufficiency were the same in the treatment arms; however, dose reductions for renal dysfunction were more common with zoledronic acid. Post-marketing surveillance has revealed multiple cases of renal failure attributed to bisphosphonate use [77].

Regulatory agencies have warned of the potential risk of nephrotoxicity associated with bisphosphonate use. For zoledronic acid, a dose adjustment is required for patients with a baseline GFR of 30–60 ml/min and the use of this drug is contraindicated in patients with a GFR of <30 ml/min. Renal function must be assessed prior to each dose of zoledronic acid and deterioration of renal function requires cessation of drug until improvement in renal function. Patients should be counseled regarding the potential nephrotoxicity associated with bisphosphonate use prior to initiation of treatment with these agents.

Other Areas for Consideration

A few additional areas merit particular attention. For one, despite the correlation of bone turnover markers with survival and disease progression, the levels of bone turnover markers are not yet used to guide clinical decision making [42]. Therefore, there is no clear role for monitoring bone turnover markers during therapy. There is limited data available with regard to switching from one osteoclast inhibitor to another. In one randomized phase II study patients with bone metastases from multiple solid tumor types elevated uNTx level despite treatment with a bisphosphonate were randomized to remain on the bisphosphonate or to treatment with denosumab [78, 79]. Denosumab was more effective at reducing uNTx and there were no signals of excessive toxicity upon switching between classes of osteoclast inhibitor. Though not powered to investigate a decreased incidence of SREs, a lower proportion of patients in the denosumab group (3 vs. 19 %) developed an SRE during this study. Despite these data, a phase III trial would be required to explore the safety and efficacy associated with switching from one osteoclast inhibitor to another.

Despite treatment with a bone-targeted agent many patients with mCRPC will ultimately develop an SRE. The continuation of zoledronic acid beyond an on-study SRE has been shown to delay the onset of a second SRE [80]. Therefore, following the occurrence of an SRE in a patient with mCRPC on treatment with an osteoclast inhibitor, it is prudent to continue a bone-targeted agent.

The natural history of advanced prostate cancer is typified by a period of sensitivity to castration followed by the development of castration-resistant disease. Patients diagnosed with metastatic castrate-sensitive disease have a median survival of greater than 5 years [81]. The registration trial exploring the use of denosumab in metastatic prostate cancer included only patients with mCRPC. Therefore, there is essentially no data on which to determine the safety and efficacy of denosumab in patients with metastatic castrate-sensitive prostate cancer. It appears that longer duration of therapy with bisphosphonates may be associated with an increased risk of ONJ [82]. The median onset of ONJ associated with denosumab or zoledronic acid is after 14 months of treatment [56, 70]. Therefore, although ONJ is a relatively uncommon complication of osteoclast inhibition, it is likely that a portion of patients with castrate-sensitive disease will develop ONJ prior to castration-resistance when disease is more difficult to control. As progression of disease is a major risk factor for ONJ [66], these patients may not be candidates for further treatment with an osteoclast inhibitor in the setting in which they would be most likely to benefit from a bone-targeted agent. Therefore, as the benefit of initiating an osteoclast inhibitor for prevention of SREs in castrate-sensitive disease is not yet established, it is reasonable to initiate an osteoclast inhibitor at castration-resistance. Special considerations may also be appropriate for patients with castrate-sensitive disease that have experienced an SRE.

The most appropriate duration of treatment with either denosumab or zoledronic acid is not yet established. In the 103 trial the median duration of therapy with denosumab was 12.2 months and was 11.2 months with zoledronic acid [56]. In the 039 trial subjects received zoledronic acid for 24 months [50]. In breast cancer, after 5 years of therapy with denosumab or zoledronic acid the incidence of ONJ is 4.7 and 3.5 %, respectively [83]. Given the comparatively low incidence of ONJ relative to the incidence of SREs in patients with mCRPC, it is reasonable to continue treatment with an osteoclast inhibitor indefinitely in the absence of adverse events such as ONJ.

Conclusion

Advanced prostate cancer is associated with significant skeletal morbidity both from fragility fractures in patients requiring ADT and in the form of SREs in patients with CRPC metastatic to bone. Much insight has been gained with regard to both normal bone physiology and the pathophysiology that accompanies advanced disease.

Osteoclast inhibition with either bisphosphonates or denosumab has become routine in the treatment of advanced disease. Other recent advances in the treatment of mCRPC include the introduction of a number of active agents shown to extend survival. Bone-targeted agents and recently approved disease modifying agents will contribute to improved outcomes in advanced prostate cancer. Osteoclast inhibitors are associated with a generally favorable adverse event profile, but a number of potential adverse events such as ONJ and hypocalcemia (and potential nephrotoxicity with bisphosphonates) deserve particular attention both to reduce incidence and to mitigate potential morbidity.

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Radium-223 and Other Radiopharmaceuticals in Prostate Cancer

11

Oliver Sartor and Brian Lewis

History of Radium

The discovery of electrically generated X-rays by William Röntgen in 1895 was a seminal discovery that heralded a series of subsequent discoveries related to radiation and radioactivity [1]. In 1896 Henri Becquerel described rays similar to X-rays derived from uranium salts [2]. Marie Sklodowska Curie and her husband Pierre examined various forms of uranium and made the conclusion that the emissions came from the atom. As Marie Curie later stated, "I then began to investigate the different known chemical elements, to determine whether there exist others, besides uranium, that are endowed with atomic radioactivity—that is to say, all the compounds of which emit Becquerel rays." [3] After a systematic search for other compounds capable of emitting Becquerel rays, the Curies discovered such emissions from thorium as well.

Her attention then turned to "pitchblend," a complex radioactive blend that contained uranium ores but which had even more emissions than uranium, thus suggesting that elements other than uranium were responsible for the pitchblend radioactivity. From pitchblend mixture, the Curies were able to identify both radioactive polonium and then radioactive radium. Radium was first described by the Curies in 1898 [4] but not purified in metallic form until nearly a decade later. Marie and Pierre Curie coined the term "radioactivity." Henri Becquerel, Marie Curie, and Pierre Curie were awarded the Nobel Prize in 1903. That same year, Marie Curie was also awarded her doctorate degree. After the death of her husband in an accident, Marie Curie working alone isolated pure, metallic radium and was the recipient of the 1911 Nobel Prize in chemistry "in recognition of her services to the advancement of chemistry by the discovery of the elements radium and polonium, by the isolation of radium and the study of the nature and compounds of this remarkable element." [5] She was the first person to win a second Nobel Prize and the first women to serve as a Professor at the Sorbonne.

Early Anti-cancer Work with Radium

The exact first application of radium in cancer therapeutics is difficult to ascertain. It is clear from a 1903 article published in the British Medical Journal [6] that multiple applications of radium in anti-cancer treatment were ongoing shortly after the description of radium's radioactivity. A 1903 report from Vienna indicated that radium salts applied locally could destroy a tumor of the palate that had been resistant to all alternative therapy. These salts were predominantly the radium-226 isotope, the most common radium isotope.

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F. Saad and M.A. Eisenberger (eds.), *Management of Castration Resistant Prostate Cancer*, Current Clinical Urology, DOI 10.1007/978-1-4939-1176-9_11,

The radium treatments in that era involved either direct application or interstitial seeding and thus represented the beginning of brachytherapy as an anti-cancer approach [7].

Bone Targeting of Radium and Other Radiopharmaceuticals

The alkaline earth metals in the periodic table contain a series of elements including calcium (Ca), strontium (Sr), barium (Ba), and radium (Ra) (see Fig. 11.1). Each of these compounds are, to various degrees, calcium-mimetic in the human body and each of these agents can deposit in osteoblastic bone metastatic sites. Samarium-153 (Sm-153) binds to bone by virtue of its chelation to a compound termed ethylenediaminetetramethylenephosphonic acid (EDTMP).

For the bone-targeted radiopharmaceuticals such as Sm-153 EDTMP, strontium-89 (Sr-89), radium-223 (Ra-223), and phosphorus-32 (P-32), as well as the bisphosphonates, bone targeting occurs via hydroxyapatite Ca5(PO4)3(OH) binding [8]. Hydroxyapatite is an essential portion of the inorganic matrix of bone and is inter-mixed with cancer cells in lesions with an osteoblastic phenotype. Targeting radioactive compounds to hydroxyapatite is also performed clinically when using technetium-99 linked methylene diphosphonate (MDP) bone scans or sodium fluoride (F18) bone scans. When imaging bone lesions with either of these agents, the actual radiopharmaceutical complex is within the hydroxyapatite within the regions of interest.

Beta-Emitting Radiopharmaceuticals: A Brief Overview

The first use of a bone-targeted radiopharmaceutical involved phosphorus-32 (P-32). In 1950, it was reported by Friedell and Storaasli [9] that bone metastatic breast cancer could be palliated after treatment with intravenous P-32. The first use of a radiopharmaceutical in bone-metastatic prostate cancer was reported by Maxfield, Maxfield, and Maxfield in 1958 [10]. Though efficacious, P-32 is rarely used today in the United States in part due to myelosuppression.

Three bone-targeted radiopharmaceuticals are currently FDA approved and in common use in the United States. These include Sr-89, Sm-153-EDTMP, and Ra-223. The strontium and samarium radionuclides are beta particle emitters

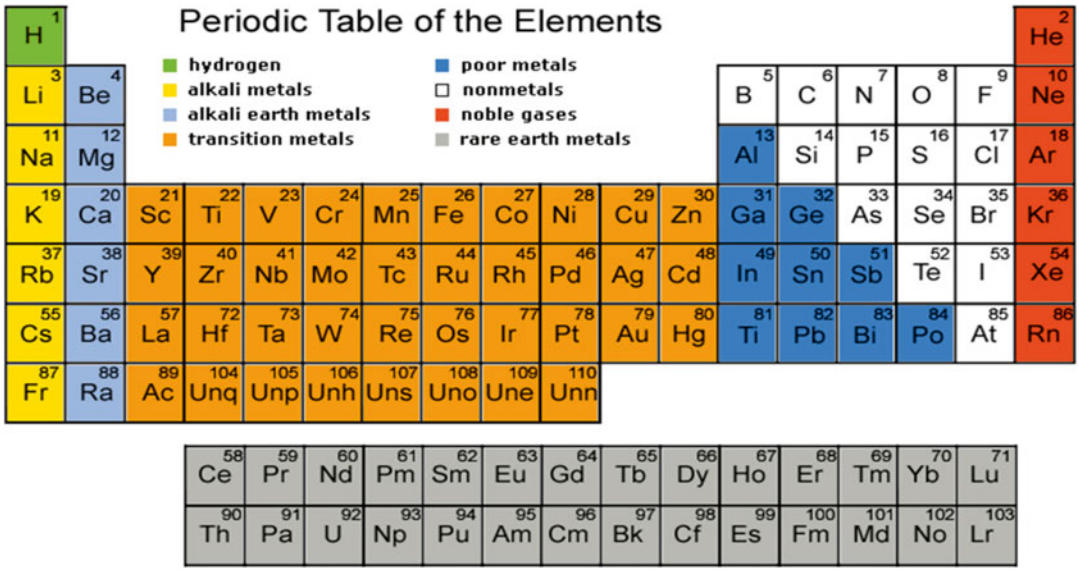

Fig. 11.1 Periodic table

but the radium nuclide is an alpha particle emitter.

Sr-89 beta particles are emitted with a higher energy as compared to Sm-153 [11]. The tissue penetration of the particles is proportional to energy, thus the particles emitted by Sr-89 are penetrative than those of Sm-153. For Sr-89, the average beta energy is 0.580 MeV. For the Sm-153, the average betas are 0.23 MeV. The tissue penetration is approximately 2.4 mm for betas derived from Sr-89 and approximately 0.5 mm for betas derived from Sm-153. The Sr-89 half-life is 50.5 days. The half-life for Sm-153 is approximately 1.9 days. Sm-153 has an energetic gamma emission which allows this agent to be used for imaging as well. A placebo-controlled randomized trial was pivotal for FDA approval of Sr-89. A total of 126 metastatic castrate-resistant prostate cancer (CRPC) patients were randomized to intravenous Sr-89 at a dose of 10.8 mCi or an intravenous placebo [12]. A higher percentage of patients in the strontium arm stopped analgesics 3 months after injection (17 versus 2 %). New painful bone lesions were also tracked prospectively. Three months post-injection, 59 % of patients in the placebo arm had new painful lesions compared to 34 % of Sr-89 treated patients. Grade 4 low platelets were noted in 10.4 % of Sr-89 treated patients.

Two randomized placebo-controlled trials were pivotal for the FDA approval of Sm-153 EDTMP in painful bone-metastatic CRPC patients. The first trial [13] utilized a heterogenous group of bone-metastatic cancer patients, but nearly two-thirds had prostate cancer. Patients were randomized to Sm-152 (non-radioactive) combined with EDTMP or Sm-153-EDTMP at two doses (0.5 mCi/kg or 1 mCi/kg). The trial was double blinded and utilized pain as a primary endpoint. A total of 72 % of patients randomized to receive 1.0 mCi/kg Sm-153-EDTMP had pain relief within or at 4 weeks of treatment. Thrombocytopenia and leukopenia decreased to a grade 3 level in 3 and 14 % of patients in this radionuclide arm but recovery by 8 weeks was typical.

A second Sm-153-EDTMP placebo-controlled randomized trial [14] was conducted in 152 bone metastatic CRPC. Patients were randomized 2:1 to receive a 1.0 mCi/kg dose of the radiopharmaceutical of or placebo. Pain relief was the primary endpoint. A reduction in pain and analgesic consumption was noted 3–4 after the injection and 38 % of patients treated with Sm reported complete pain relief compared to 18 % of patients treated with placebo. Grade 3 decreased in platelets and white cells were documented in 3 and 5 % of Sm patients, respectively.

Distinctions Between Alpha and Beta Particles

Both Sr-89 and Sm-153 are beta-emitting isotopes FDA approved for treatment of patients with bone-metastatic lesions. Beta particles are basically electrons emitted from the nucleus of these radionuclides. Alpha particles, such as those derived from Ra-223, represent a novel concept in medicine. Alpha particles are two neutrons and two protons, and have a mass approximately 7,300-fold higher than beta particles. The energy of alpha particles is not proportional to mass as particle velocity varies. Beta particles velocity is approximately 90 % of the speed of light whereas alpha particles velocity is approximately 5–10 % of the speed of light [15, 16]. Particle energies of various isotopes are catalogued in Table 11.1.

Alpha particle emitters have the ability to deliver radiation to a highly localized region. Alpha particles have a tissue range typically of only a few cell diameters, 40–100 μm. Given the small radius of delivery, normal cells are unlikely to be within the radiation crossfire assuming that the alpha-emitter can be delivered specifically to the region containing the cancer. The alpha particle linear energy transfer (LET) is typically in the range of 25–230 kEv/μm, which is 100–1,000 fold higher than the average beta particle LET. Higher LETs results in more cellular damage. The combination of the short range alpha particle, and the high LET, generates a highly focal but highly cytotoxic dose of radiation therapy.

Table 11.1 Physical properties of the selected radiopharmaceuticals

Radionuclide	Half-life (days)	Main particle	Maximum energy (MeV)	Mean energy	Average penetration (mm)
Radium-223	11.4	Alpha	27.78	6.94	<0.1
Strontium-89	50.5	Beta	1.46	0.58	2.4
Samarium-153	1.9	Beta	0.81	0.22	0.5
Phosphorus-32	14.3	Beta	1.71	0.69	3.0
Yttrium-90	2.7	Beta	2.27	0.93	4.0
Lutetium-177	6.7	Beta	0.49	0.14	0.3
Iodine-131	8.0	Beta	0.61	0.19	0.8
Rhenium-186	3.8	Beta	1.07	0.33	1.0
Rhenium-188	0.7	Beta	2.12	0.64	3.8
Holmium-166	1.1	Beta	1.84	0.67	3.3
Tin-117m	13.6	CE	0.15	0.14	0.2

Double strand breaks in cellular DNA are considered the most relevant effect of radiation by alpha particles. The DNA double strand breaks cause impairment of cellular division and can trigger apoptosis. The double strand breaks are also more resistant to DNA normal repair mechanisms and thus are more lethal than the single strand breaks caused by other radiation modalities [17].

Radium-223 Decay

Radium is an elemental chemical in the alkaline earth metal family (along with calcium and barium and strontium). It has multiple known isotopes with Ra-226 being the most common and the longest lived with a half-life of 1,601 years [18]. Ra-223 (which the rest of this paper will discuss) on the other hand has a half-life of only 11.4 days which makes it more applicable for medicinal purposes. Ra-223 decays to lead-207 after emitting 4 alpha particles through the following decay chain: Ra-223 (half-life of 11.4 days) to radon-219 (half-life of 3.96 s) to polonium-215 (half-life of 1.78 ms) to lead-211 (half-life of 36.1 min) to bismuth-211 (half-life of 2.17 min) to thorium-207 (half-life of 4.77 min) to lead-207 (stable). Several weak gamma emissions and a low energy beta are also present in the Ra-223 decay chain.

Initial Human Studies with Radium-223

The first clinical experience with Ra-223 was in a phase I trial with a single injection of Ra-223 with 5 patients in each of the following doses, 46, 93, 163, 213, and 250 kBq/kg [19]. Both breast (n = 10) and prostate cancer (n = 15) patients were included in the trial. All patients had bone-metastatic disease. Palliative responses were evaluated via the QLQ-C30 questionnaire, a patient reported pain scale. Pharmacokinetics were performed after intravenous radium-administration. Weekly blood sampling indicated that there was a mild and reversible suppression of bone marrow function with a nadir 2–4 weeks after the injection. Thrombocytopenia was only noted at grade 1 levels. Grade 3 leukopenia was noted in three patients, one patient at each of three higher Ra-223 dose levels. No dose limiting myelosuppression was observed. No patient dosed at the 46 and 93 kBq/kg doses had grade 3 leukopenia and no patient at the two lower doses had any thrombocytopenia. A transient diarrhea was observed in 10 of the 25 patients and was not clearly dose related. A bone pain typical of "flare" was noted in 9 out of 25 patients. Vomiting was noted in 5 of 25 patients and nausea was noted in 5 of 25 patients. In the highest dosage group, 4 out of 5 patients reported some degree of both nausea and vomiting.

Radioactivity levels in the blood 10 min post-injection were 12 % of the initial estimated values; at 1 h levels were 6 % and after 24 h, reduced to <1 %. Excretion of Ra-223 was primarily intestinal. Ra-223 deposition and excretion can be imaged given the weak gamma emission previously noted in the decay chain. The imaging quality is not high, however, because of the low amount of gamma emissions.

All patients had a decline in serum alkaline phosphatase (ALP) levels after Ra-223 in the phase I trial; the mean decrease was more pronounced in the patients with prostate cancer (52.1 %) versus the breast cancer patients (29.5 %). There was a pain score improvement in a number of patients but symptom relief conclusions were limited by the lack of a placebo control group assessment. For pain, at the 1-week time point, 52 % had pain improvement, 36 % were not changed, and 12 % worse pain. At the 4-week point, 60 % reported improvement, 20 % were not changed, and 20 % had a worse pain. At the 8-week point, 56 % reported improvement, 24 % were not changed, and 20 % had a worse pain. There was no clear evidence for dose responsiveness in the pain scores. Taken together, these results encouraged further study.

A randomized phase II placebo-controlled multi-institutional study of Ra-223 was published in 2007 [20]. In this 64 patient study, patients with castrate-resistant and bone-metastatic prostate cancer who needed external-beam radiation therapy for pain relief were assigned to four intravenous injections of either Ra-223 at a dose of 50 kBq/kg or placebo. The primary endpoints were changes in bone ALP levels and time to initial skeletal-related events (SREs). SREs were unconventionally defined as including radiation or surgery to bone, increases in bone pain, increases in analgesic consumption secondary to skeletal pain, initiation of treatments for skeletal progression, or neurologic consequences derived from skeletal metastasis. Secondary endpoints included assessments of safety, various serum markers of bone turnover including total ALP, procollagen I N-propeptide (PINP), C-terminal crosslinking telopeptide of type I collagen (CTX-1), and type I collagen crosslinked C-telopeptide (ICTP). Serum PSA and overall survival were also assessed.

This is an unequivocal decrease of median bone ALP of nearly 66 % in the Ra-223 group as compared to the placebo group which has a median increase of 9.3 %. Compared with the placebo group, the Ra-223 treated patients had a statistically significant reduction in all markers of bone turn-over (bone-ALP, total-ALP, PINP, CTX-I, and ICTP). The median time to first SRE was 14 weeks in the Ra-223 group and 11 weeks in the placebo group ($P = 0.257$). The medium time to PSA progression was improved in the radium group as compared to placebo (26 versus 8 weeks). The median overall survival had a strong trend toward improvement in the radium treated group ($P = 0.066$) with median survival of 65.3 weeks in the radium arm and 46.4 weeks for the placebo arm. The toxicity was relatively minimal in this trial with constipation being more common in the radium as compared to the placebo group. These trials lead directly to the planning and execution of the phase III Ra-223 trial (see Fig. 11.2).

Radium-223 Phase III Trial

Building upon the phase I and II experience, a phase III placebo-controlled international randomized double-blind controlled trial (ALSYMPCA) was designed to test whether or not Ra 223 would prolong survival in patients with bone-metastatic castrate-resistant prostate cancer [21]. The inclusion and exclusion criteria are key to understanding these trial results, these factors included the following:

1. Patients were required to have at least two bone metastatic lesions as assessed by a conventional bone scan;
2. No visceral metastases were allowed;
3. Some degree of symptoms were required (either regular use of any analgesic medication or treatment with an external-beam radiation therapy for cancer-related bone pain within the prior 12 weeks);
4. A minimum PSA of 5 ng/ml and an ECOG performance status of 0–2;

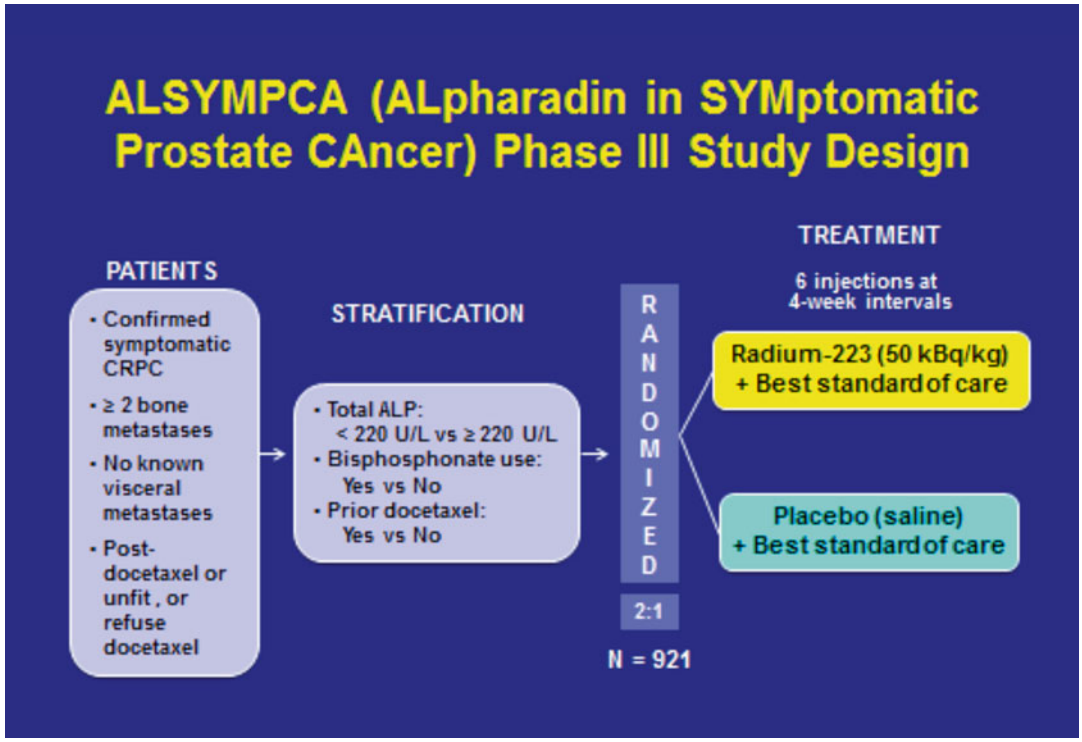

Fig. 11.2 Schematic of the phase III radium-223 trial

5. Life expectancy of 6 months or longer;
6. Prior treatment with docetaxel, refuse docetaxel, or be unfit for docetaxel;
7. Adequate hematologic, renal, and liver function;
8. No patient could have had prior hemi-body radiation, systemic radiotherapy with another isotope within the prior 24 weeks;
9. No patient could have impending spinal cord compression.

At the time of randomization, patients were stratified according to three criteria including prior use (or no prior use) of docetaxel, a baseline ALP of less than or greater than 220 units per liter, and current use (or nonuse) of a bisphosphonate. Zoledronic acid was the predominant bisphosphonate in use for those patients taking a bisphosphonate.

The primary endpoint of this study was overall survival and a variety of secondary endpoints including time to first symptomatic skeletal event (SSE), ALP declines, and PSA declines, were prospectively evaluated. Symptomatic skeletal events were defined as radiation to bone, surgery to bone, pathologic fracture, or spinal cord compression. No radiographs were scheduled during the phase III trial, consequently all SSEs were clinically apparent.

The dosing scheme was interesting in that 50 kBq/Kg intravenous (IV) doses given q 4 weeks for a total of four doses were utilized in the phase II study but in the phase III trial, a total of six IV doses were planned. No prior trial of Ra-223 had included six doses of the radiopharmaceutical. The Ra-223 arm was compared to a placebo arm with a 2:1 randomization schema (see Fig. 11.2). Importantly, best standard of care treatments were allowed in both arms of the trial. The best standard of care could include a variety of hormonal agents including anti-androgens, corticosteroids, ketoconazole, various estrogens, or external-beam radiotherapy. Chemotherapy, hemi-body radiation, experimental agents, or other systemic radiopharmaceuticals were not allowed during the course of treatment.

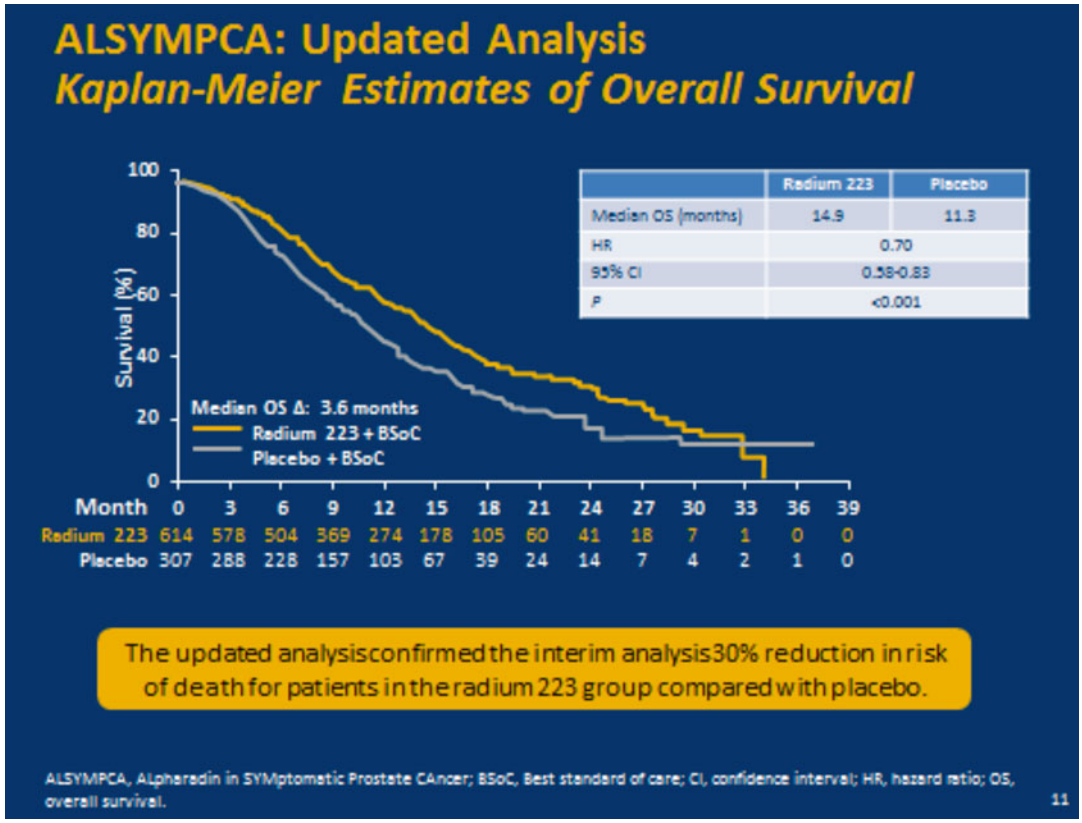

Fig. 11.3 Final results of the survival analysis in the phase III trial of radium-223

A total of 921 patients were enrolled on the trial. The trial was stopped at a formal interim analysis by the independent data monitoring committee after approximately 50 % of the planned deaths had occurred. The trial stoppage occurred as consequence of meeting the predefined endpoint for overall survival.

A total of 314 deaths had occurred at the time of the initial interim, the *P* value at the time of interim analysis for overall survival was 0.002 in favor of Ra-223, with a hazard ratio of 0.70 (95 CI 0.55–0.88). A subsequent more mature analysis (after 528 deaths) was done prior to crossover of placebo patients to active treatment. This analysis had a median overall survival advantage with a *P* value of <0.001 and a hazard ratio of 0.70 (95 CI 0.58–0.83). In the updated analysis the median overall survival was 14.9 months in the Ra 223 group and 11.3 months in the placebo group (see Fig. 11.3).

A multivariate analysis indicated that randomization to radium, higher baseline PSA, higher ALP, higher LDH, higher age, lower albumin, and lower ECOF performance status were significantly associated with a diminished overall survival [22].

It is important to note that in the pre-specified stratified analysis that patients had a longer survival whether or not they were being concurrently treated with bisphosphonate or docetaxel and the findings in those patients pre- or post-docetaxel were particularly important given that this is the first trial to analyze docetaxel use in this manner. The median survival in non-docetaxel pre-treated patients was 11.5 months in the placebo arm as compared to 16.1 months in the Ra-223 treated arm. For post-docetaxel patients, the median survival was 11.3 months in the placebo arm and 14.4 months in the Ra-223 arm. These findings were critical as regulatory

authorities have now approved Ra-223 without regard to prior docetaxel use.

All these secondary endpoints were positive including the time to first SSE. The median time to first SSE in the Ra-223 arm was 15.6 months as compared to 9.8 months in the placebo arm (HR 0.66, $P<0.0001$). The utilization of an SSE endpoint was unique. As noted, no radiographic assessments were evaluated in the phase III trial. Thus, all events that fit into the SSE category were clinically relevant. A 30 % or greater reduction in PSA blood levels of week 12 was achieved in 16 % of patients in the Ra-223 arm and in 6 % of the patients in the placebo group. A portion of the PSA decline is likely attributable to the use of various hormonal therapies in addition to the Ra-223. ALP was consistently suppressed after radium treatments, and may serve as biomarker for Ra-223 action. The mean decline in baseline in ALP at 12 week was 32 % for the radium treated patients as compared to a 37 % increase in ALP for placebo treated patients. Ra-223 significantly prolonged median time to ALP progression as compared to placebo; median: 7.4 versus 3.8 months ($P<0.0001$).

With regard to safety issues, there was very little myelosuppression. Anemia was essentially identical between the placebo and radium groups. Grade 3, 4, or 5 thrombocytopenia was seen in 7 % of the Ra 223 patients as opposed to 3 % of the placebo patients. Grade 3, 4, or 5 neutropenia was seen in 3 % of the radium patients as opposed to 1 % of the placebo patients. Imbalances in clinical adverse events included vomiting being present in 18 % of the radium patients versus 14 % of the placebo patients. Diarrhea was noted in 25 % of the radium patients as opposed to 15 % of the placebo patients. Bone pain was less frequently documented in the radium arm. The remainder of the clinical adverse events was well balanced between arms, indicating that there was a minimal effect of Ra 223 on the vast majority of measured adverse events.

Ra 223 received FDA approval on May 15, 2013 for the treatment of patients with bone-metastatic symptomatic castrate-resistant prostate cancer without visceral metastases [22]. The practical application of radium involves administration by a physician licensed in the administration of radiopharmaceuticals. Typically, radiation oncologists or nuclear medicine physicians are qualified.

It is important to note that Ra-223 was not given as monotherapy in the phase III trial but the "best standard of care" was utilized in all patients, both those who received placebo and those who received Ra-223. This allowed patients to be treated with standard therapies as chosen by their physician rather than the usual restrictions that might apply in many clinical trials. In the phase III trial, a variety of hormonal therapies can be utilized in combination with Ra-223 and no safety issues were ascertained. Though agents such as abiraterone and enzalutamide were approved after the phase III radium trial was started, that there have been no reported safety issues concerning the use of these agents in combination with Ra-223. A future phase III trial of Ra-223 is planned with abiraterone +/− radium.

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Part III

New Drug Development: Clinical Trials Design in CRPC

Introduction

12

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Introduction

Although prostate cancer is a major cause of morbidity and mortality in Western populations ([1], p. 289), clinical research into castration-resistant prostate cancer (CRPC) has only flourished within the past decade. Regarded by many as a cancer of old men, until 2004 the management of advanced prostate cancer following progression on systemic castration consisted of supportive care alone. However, the successful development of docetaxel chemotherapy showed clinicians that survival gains were possible, while the more recent novel hormonal therapies abiraterone acetate (abiraterone) and enzalutamide proved that the androgen receptor (AR) remained a key driver in CRPC. Multiple new pathways and targets are now being evaluated as potential therapeutic avenues and drug discoveries continue apace. Although significant progress has been made in the last years with the approval of several new drugs, combination strategies have thus far failed to improve overall survival (OS) in large Phase III trials. Both our successes and our many failures demonstrate the importance of intelligent clinical trial design in CRPC. From the development of early phase studies with integration of biomarker development, to Phase III design with relevant clinical and palliative endpoints and strong statistical plans that ensure that identified targets are achieved are key to streamlining the drug development process. In this chapter we will outline some of the common issues in CRPC drug development, prior to addressing them in greater depth in the following chapters.

Successful Drug Development in the Last Years

Docetaxel became a standard treatment option for men with CRPC after the TAX-327 trial was reported in 2004 and demonstrated a median survival gain of 2.9-months (hazard ratio; HR 0.76, $p=0.009$) compared to the comparator of mitoxantrone, as well as improving pain control and quality of life [2, 3]. Subsequently, cabazitaxel chemotherapy was tested in the second-line setting after docetaxel and improved survival by a median of 2.4 months (HR 0.7, $p<0.0001$) compared to mitoxantrone [4].

The novel hormonal agents abiraterone and enzalutamide have been registered both in the

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F. Saad and M.A. Eisenberger (eds.), *Management of Castration Resistant Prostate Cancer*, Current Clinical Urology, DOI 10.1007/978-1-4939-1176-9_12,

US and Europe [5–7]. For the CYP17 inhibitor abiraterone, a total of three Phase I/II and three Phase II trials were performed, enrolling 234 patients. These trials tested safety and tolerability and included pharmacodynamic and novel activity markers [8, 9]. The subsequent randomized Phase III trials demonstrated survival and clinical benefit in men with CRPC [5, 6]. The Phase I/II trial of enzalutamide included 140 patients and expanded at several dose levels in parallel to the dose escalation [10], rapidly providing extensive safety and pharmacodynamic data and strongly supporting the subsequent positive randomized Phase III trials [7].

Additional survival-prolonging treatments in CRPC [12] have included the immune therapy sipuleucel-T and the radionuclide radium^{223} [15]. A combined analysis of two small randomized trials of sipuleucel-T produced an unexpected finding of improved survival [11], a result that was subsequently confirmed in a more appropriately powered Phase III trial (median survival improvement 4.1 months, HR 0.78, $p=0.03$) [12]. Although immune responses were observed in patients receiving sipuleucel-T, PSA and soft-tissue responses were unchanged, as was time to disease progression. Mistrust of intermediate immunotherapy activity markers and methodological concerns about the placebo treatment have continued to cause concern in the oncology community [13]. In contrast, clinical development of the alpha-emitting radionuclide radium^{223} included robust demonstration of bone-activity endpoints in early studies, prior to demonstrating a median 3.6-month improvement in survival (HR 0.7, $p<0.001$), along with improvements in quality of life endpoints [14, 15].

The ClinicalTrials.gov website lists more than 100 clinical trials currently recruiting men with CRPC. Currently, about 40 % of Phase III oncological trials achieve a positive primary endpoint, suggesting that many Phase II trials provide insufficient information ([16], p. 1093). The number of previous negative CRPC trials is difficult to quantify, due in part to the well-described publication bias against reporting of negative studies, including even large randomized Phase III trials [17]. Nevertheless, it appears that research in CRPC is now flourishing and it is hoped that more therapies may soon be available to men with CRPC.

Lessons Learned from Docetaxel-Combination Trials

When the TAX327 Phase III docetaxel trial was published in 2004 it was thought to be a benchmark that could be relatively easily improved by combining docetaxel chemotherapy with a novel targeted agent [3]. More than 40 Docetaxel Phase II combination trials have been published so far, with some suggesting the potential to increase antitumor activity with acceptable toxicity [18]. However a total of eight docetaxel-combination trials have now failed to meet their primary endpoints (see Table 12.1) [40], while one arm of a ninth trial (TRAPEZE) has met the primary endpoint of improvement in bone-clinical progression-free survival (PFS) ([39], p. 371), with a reported improvement of 1 month over standard treatment. Why did all these docetaxel-combination trials fail? Table 12.1 summarizes the early Phase I/II results of the subsequently performed Phase III combination trials: in five of the nine trials Phase I data testing the combination in patients with CRPC were not available; in the trials with prior Phase II clinical data the statistical plan was not indicated or the primary endpoint was not met. Additionally, the early clinical combination trials failed to include pharmacodynamic studies.

The results of the Phase III trials were not only disappointing because they were negative but in the cases of lenalidomide, GVAX, and calcitriol the median OS was significantly worse in the combination arm compared to standard docetaxel plus prednisone and for bevacizumab and aflibercept increased rates of toxicity and treatment related deaths were reported. The lessons learnt from the negative Phase III docetaxel-combination trial are mainly that more time and effort should be spent on the design and conduct of the early Phase I/II trial and to have a clear activity signal based on a biological rationale before launching into large and costly Phase III trials.

Table 12.1 Summary of Phase III Docetaxel Combination Trials

Targeted treatment combined with docetaxel	Phase I docetaxel combination trial in CRPC, N	Any unexpected safety signals?	Pharmacodynamic studies	Phase II docetaxel-combination trial in CRPC design (N)	Statistical plan	Primary and secondary endpoints	Outcome	Comments	Phase III docetaxel combination design (N)	Statistical plan	Primary and secondary endpoints	Outcome	Comments	References
Bevacizumab	NA			Single arm phase II, N = 20	No sample size calculation done	Response (biochemical and radiological). Secondary endpoints: Toxicity, PFS, OS	11/20 (55 %) confirmed PSA declines ≥ 50 %. 3 PR	Docetaxel 60 mg/m^2, Bevacizumab 10 mg/m^2	Randomized double blind placebo-controlled N = 1,050	86 % power to detect an increase in mOS of 24 (DP + B) vs 19 m (DP), alpha 0.05	OS, secondary: 50 % PSA decline, PFS, ORR, toxicity	mOS 22.6 (DP + B) vs 21.5 m (DP), HR 0.91, (95 % CI 0.78-1.05)	Increased treatment related deaths 4 vs 1.2 % $p = 0.05$; PFS improved 9.3 vs 7.5 m $p < 0.001$; ORR improved 49.4 vs 35.5 %, p 0.0013	[19, 20]
Aflibercept	Phase I combination trial with docetaxel in solid tumors (N = 54), N prostate cancer pts included NA, no objective responses in prostate cancer [21]; Phase I/II study in ovarian, peritoneal, and fallopian tumors, no prostate cancer included [22]			NA					Randomized double blind placebo-controlled N = 1,224	Powered to detect a HR of 0.8 with 90 % power, alpha 0.044	OS, secondary: PFS, PSA PFS, ORR, safety, pain response, pain PFS	22.1 (DP + A) vs 21.2 m (DP), HR 0.94, (95 % CI 0.82-1.08)	Significantly more G3-4 GI-toxicity (30 vs 8 %), hemorrhagic events (5.2 vs 1.7 %) and treatment related fatal events (3.4 vs 1.5 %)	[21–23]

(continued)

Table 12.1 (continued)

Targeted treatment combined with docetaxel	Phase I docetaxel combination trial in CRPC, N	Any unexpected safety signals?	Pharmacodynamic studies	Phase II docetaxel-combination trial in CRPC design (N)	Statistical plan	Primary and secondary endpoints	Outcome	Comments	Phase III docetaxel combination design (N)	Statistical plan	Primary and secondary endpoints	Outcome	Comments	References
Atrasentan	N = 12	Increased rate of hematological toxicity (neutropenic fever) in combined phase I/II study (21 %)	Bone alkaline phosphatase, urinary N-telopeptides	N = 19 (expansion cohort)	Max. 24 pt expansion cohort, improvement of PSA response rate from 40 to 65 %, type I error 0.05, power 0.86	PSA decline, Soft-tissue response	Confirmed PSA declines ≥50 % in 22.5 % pts, confirmed PSA declines ≥30 % in 35 % pts. Soft-tissue responses in 2/13 pts (15 %)	Prednisone was not given in combination with docetaxel and may account for lower PSA decline rates. MTD was not clearly defined, a dose of docetaxel of 70-75 mg/m^2 was recommended. Overall survival 17.6 m (19.2 in TAX327 study)	D vs D + Atrasentan, 1:1; N = 994	25 % increase in PFS (6.0 vs 7.5 m) and in OS (18 vs 22.5 m)	Co-primary endpoints PFS and OS	Trial halted for futility after third interim analysis. PFS 9.2 vs 9.1 m (HR 1.02, 95 % CI 0.89–1.16). OS 17.8 vs 17.6 m (HR 1.04, 95 % CI 0.90–1.19)	Atrasentan monotherapy Phase III trial in CRPC did also not meet the primary endpoint	[24–26]
Zibotentan	N = 6	No DLTs, all 3 pts in the 15 mg Zibotentan group had ≥ G3 AE (G3 neutropenia, G3 hypertension, G3 gastrointestinal hemorrhage)	NA	Randomized phase II, N = 31 (20 DP + Zibotentan vs 11 DP)	Increase in PSA response rate from 45 % (TAX327) to 65 %, alpha 0.2, power 75 %	PSA response	PSA response 85 vs 73 %, ORR 22 vs 17 %	Urinary NTx stable in D + Zibotentan group, bone alkaline phosphatase and procollagen type I N propeptide (PINP) reduced in both groups. c-Terminal cross-linking telopeptides unchanged.	Randomized double blind placebo-controlled N = 1,052	Powered to detect a HR of 0.75 with 90 % power, alpha 0.05	OS, secondary endpoints: pain PFS, PFS, SRE, PSA PFS and PSA response rate	mOS 20 vs 19.2 m (HR 1.00, 95 % CI 0.84-1.18)	No differences in secondary endpoints. Cardiac failure 5.6 vs 1.7 %	[27, 28]

Dasatinib	N = 16	No DLTs, MTD not reached	Urinary N-telopeptide, bone alkaline phosphatase	N = 30 (single arm phase II)	Not given	Not given	PSA declines ≥ 50 % for ≥ 6 in 26 of 46 patients (57 %). Soft-tissue partial responses 18/30 evaluable pts (60 %)		Double blind, placebo-controlled, 1:1; D + P vs D + P + Dasatinib; N = 1,522	NA	OS; ORR, time to first skeletal-related event (TFSRE), time to prostate-specific antigen progression (TPSAP), urinary N-telopeptide (uNTX) reduction, pain reduction, progression-free survival (PFS), and safety	21.5 vs. 21.2 m; HR 0.99; $p = 0.90$	Modest delay in SREs	[29, 30]
GVAX	No docetaxel combination study, monotherapy in CRPC Phase I/II (N = 80)			NA					N = 600	NA	OS	12.2 (DP + GVAX) vs 14.1 m (DP) (HR 1.7, 95 % CI 1.15-2.53)	Trial terminated early due to an imbalance in deaths in the experimental arm.	[31, 32]
Lenalidomide	N = 34	G4 neutropenia (2 pts) and febrile neutropenia (1 pt)	NA	NA				Not performed	Double blind, placebo-controlled, 1:1, D + P vs D + P + Lenalidomie; N = 1,059	NA	Primary: OS. Secondary PFS, ORR, safety	OS NR (D + P) vs 17.8 m (D + P + L) (HR 1.53, 95 % 1.17-2.0), PFS NR (D + P) vs 10.4 m (D + P + L), (HR 1.32, 95 % CI 1.05-1.66)	Median number of treatment cycles 8 (D + P) vs 6 (D + P + L), rate of febrile neutropenia 12 %	[33, 34]
Calcitriol	NA			N = 37 (Docetaxel 36 mg/m^2 for 5 weeks of a 6 week cycle, calcitriol 0.5 μg/kg)	20 % increase in PSA response rate from 40 to 60 %, alpha 0.05, power 80 %	Confirmed PSA decline ≥50 %. Secondary endpoints: ORR, PFS, OS	PSA response 81 %, ORR 53 %. mOS 19.5 m							[35]

(continued)

Table 12.1 (continued)

Targeted treatment combined with docetaxel	Phase I docetaxel combination trial in CRPC, N	Any unexpected safety signals?	Pharmacodynamic studies	Phase II docetaxel-combination trial in CRPC design (N)	Statistical plan	Primary and secondary endpoints	Outcome	Comments	Phase III docetaxel combination design (N)	Statistical plan	Primary and secondary endpoints	Outcome	Comments	References
				N=250, double blind randomized. Docetaxel 36 mg/m^2 for 3 weeks of 4 cycle, DN-101 45 µg (1 day before chemotherapy)	20 % increase in PSA response rate from 45 to 65 %, alpha 0.045, power 85 %	Confirmed PSA decline ≥50 %. Secondary endpoints: ORR, PFS, OS	PSA responses 58 vs 49 %	mOS not reached for DP+DN101 estimated 24.5 vs 16.4 m (DP), (HR 0.7, 95 % CI 0.48-1.04)	Randomized open label phase III, N=953	Powered to detect a HR of 0.78 for OS, power 90 %, alpha 0.05	Primary: OS. Secondary: safety, PFS, SRE-free survival	Trial halted for futility. OS 17.8 m (DP+DN-101) vs 20.2 m (p=0.002)	Significantly more toxicity leading to Docetaxel dose modification 31 vs 15 %	[36, 37]
Custirsen (OGX-011)	40 pts, 8 cohortsq	At OGX-011 640 mg and Docetaxel total 9 pts 3 DLTs (G4 mucositis, G3 fatigue, G4 neutropenia 6 days)	Clusterin expression in mononuclear cells decreased in all pts	Randomized phase II (N=82 pts)	90 % to detect difference in PSA response rate of 60 vs 40 %, alpha 0.1	Confirmed PSA decline ≥50 %. Secondary endpoints: ORR, PFS, OS, changes in serum clusterin	PSA declines ≥50 %: 58 vs 54 %. ORR 19 vs 25 %	mOS 23.8 vs 16.9 m (HR 0.61, 95 % CI 0.36-1.02). Median decrease in serum clusterin at end of cycle 1 in DP+OGX-011: 26 % vs increase in 0.9 % on DP alone	Double blind, NOT placebo-controlled, N=1,000	NA	OS. Secondary: PFS, safety, ORR, PSA response rate	NA	NA	[38]
Strontium +/− Zoledronic acid	NA			N=200 (4 arms: DP vs DP+Zoledronate vs DP+Sr89 vs DP+SR89+Zoledronate)	NA	Feasibility and safety	No safety issues identified, trial moved into phase III part	NA	N=757; patients randomized to six cycles of DP: alone; with ZA; with a single dose of Sr89 after cycle 6 or both	Power 90 %, alpha 0.05	Co-primary endpoints: bone-clinical PFS (SRE, death from any cause, bone pain progression). Secondary EP: OS	Clinical PFS DP+Sr89 9.8 vs 8.8 m (DP), HR 0.8, (95 % CI 0.66-0.97)	mOS DP+SR89 16.8 vs 16.4 m (DP)	[39]

The Challenges of Performing Trials in CRPC

Survival Endpoints

With the approval of several novel agents in the last 4 years, patients are likely to be exposed to several survival-prolonging treatments, impacting OS benefits from an experimental therapy tested as an early line of treatment. A median OS benefit for a novel agent may therefore no longer be an achievable endpoint. With the COU-AA-302 trial pre-chemotherapy abiraterone was only licensed based on the proven survival benefit post-docetaxel and the significant improvement in the co-primary and all secondary endpoints. However, orteronel (TAK-700) may be the first casualty of the increased access to active post-trial therapies. The changing landscape of drug development in CRPC therefore urgently requires validation of intermediate endpoints.

Surrogacy Endpoints

In order to fulfill the criteria of surrogacy a biomarker must meet several prospectively defined criteria (termed the Prentice Criteria) and must be validated across multiple trials of different anticancer treatments [41]. At present there are no surrogate endpoints for OS in CRPC. Decreases in circulating tumor cell (CTC) counts and the combination of CTCs and LDH have shown promise as survival surrogates [42, 43].

Prognostic Markers

At least 20 baseline (pre-treatment) and eight on-treatment factors have been shown to have prognostic significance in CRPC patients [44]. These factors were combined into nomograms that predicted survival for chemotherapy-naïve CRPC patients [45–48]. These nomograms were developed before the introduction of the novel treatments and require updating for contemporary CRPC populations [49].

Increasing use of molecular characterization technologies may soon allow CRPC patients to be stratified within trials, or for development of niche therapies targeting key mutations in subsets of CRPC patients. Although these approaches are likely to improve response rates, challenges of molecular heterogeneity within tumors and patients, as well as parallel signaling pathways within cancer cells, will need to be addressed moving forward.

Tissue Acquisition

Translational studies often require tissue and mandating available blocks or slides at study entry is one method for protecting the validity of these important exploratory objectives. Although prostate cancer most commonly metastasizes to bone, making biopsy more challenging, the ability to maximize opportunities for prostate cancer patients to participate in both cancer-specific and solid tumor Phase I studies should encourage clinicians to pursue tissue acquisition whenever possible.

Palliative Endpoints

The FDA has recently underlined the importance of including patient reported outcomes (PRO) in clinical trials [50]. Recommendations for the appropriate inclusion of PROs in prospective clinical trials were published in 2012 [51]. However, PRO are often included as exploratory estimations rather than being based on protocol-specified hypotheses and sound statistical plans [52]. Most recent Phase III trials in CRPC have included quality of life (QOL) measurements and pain diaries [53, 54]. However a recent Phase III trial (NCT 01083615) with a primary endpoint of durable pain control was closed early due to poor recruitment. Research into palliative endpoints such as pain control and symptomatic improvement in QOL should be encouraged especially in view of the fact that patients live significantly longer and after several lines of treatment palliation may be the key priority.

Conclusion

For many years treating patients with advanced metastatic prostate cancer was frustrating due to the lack of treatment options. Today, physicians are in a different position with six survival-prolonging treatments that have already or will soon be approved for patients with castration-resistant disease. New challenges have arisen for the drug development spectrum from Phase I to III clinical trials. The key issues for Phase I/II clinical trials include: a strong pre-clinical rationale; testing of the novel compound or combination in the relevant patient population; collecting convincing pharmacokinetic and pharmacodynamic evidence supporting further development; and demonstrating antitumor activity that merits further evaluation in an increasingly competitive environment. Key issues in Phase III clinical trial development are defining the appropriate patient population, utilizing a sound statistical plan and the careful choice of primary endpoint, particularly in the absence of intermediate endpoints and markers of surrogacy. Also with CRPC patients living longer, prevention of complications such as skeletal-related events and management of adverse events will be important. Chapters 13–15 will discuss Phase I/II trial development of targeted agents in CRPC, selection of clinical and palliative endpoints, and the issues surrounding defining targets and meeting trial endpoints.

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Phase I–II Targeted Treatments

13

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Introduction

Prostate cancer research is a burgeoning field. Multiple survival-prolonging treatments target common signaling pathways in castration-resistant prostate cancer (CRPC), while molecular analyses have identified new pathways and targets. Optimizing the clinical development of novel anticancer drugs for this disease remains an urgent priority, with several challenges pertaining to disease response assessments, demonstration of sufficient clinical benefit to acquire regulatory approval, and the acquisition of tumor tissue for predictive and pharmacodynamic studies. In this chapter we will review issues specific to conducting Phase I and II clinical trials of targeted therapies for men with CRPC. These include trial design, selection of trial participants, and the appropriate safety and efficacy monitoring whilst on trial. Overall, there is no "one size fits all" for early phase studies. Indeed, it is both appropriate and necessary that the design and efficacy measures of an immunotherapy trial differ from that of an androgen receptor (AR) targeting therapy. However, all early phase trials should strive to incorporate robust biological and scientific hypotheses, analytically validated biomarkers with known data on reproducibility and variability, detailed pharmacodynamic studies in normal and tumor tissues, as well as pursuing appropriate patient selection.

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Phase I Clinical Trials

Trial Design

Phase I studies are usually the first human in vivo trials in CRPC. The intent of such trials is primarily, to provide safety data for the compound or combination strategy and secondarily, to obtain preliminary signals of efficacy that can then be pursued with appropriately powered trials.

Dose escalation may be performed using a traditional stepwise approach, with serial patient cohorts based on the Fibonacci method, or an accelerated titration with single patient cohorts [1]. Rapid dose escalation schemes have the advantage of exposing minimal number of patients to subtherapeutic doses while preserving safety and maintaining rapid accrual [2]. Alternatively, adaptive Bayesian designs may be utilized [3],

F. Saad and M.A. Eisenberger (eds.), *Management of Castration Resistant Prostate Cancer*, Current Clinical Urology, DOI 10.1007/978-1-4939-1176-9_13,

allowing "real-time" modifications based on the accumulating information. Bayesian design trials require intensive statistical input in the design and during conduct of trials, but are postulated to be highly informative [4]. Dose escalation methods may also be important in the context of novel potent AR targeting compounds where antitumor activity may be observed at the lowest dose levels, defining a minimally efficacious dose, since this may have important safety and fiscal consequences.

Expansion cohorts are increasingly incorporated into Phase I trials, allowing further exploration of biologically active doses below the dose limiting toxicity (DLT) level. The potent antiandrogen enzalutamide was tested in this manner, moving seamlessly from a Phase I/II study [5] to the definitive Phase III trial [6]. The Phase I/II enzalutamide trial expanded several dose levels between 60 and 480 mg per day, generating Phase II clinical data at multiple dose levels in very short time and providing comprehensive data on safety and tolerability as well as information about antitumor activity in both pre- and post-docetaxel settings. The main advantage to a combined Phase I/II trial is the speed achieved by streamlining processes.

Defining the Target

An increasing number of molecular targets have been identified for drug discovery for the treatment of CRPC. The AR remains a key target in CRPC, with the recent registration of abiraterone acetate (abiraterone) and enzalutamide [6–8]. Due to the very high expression of AR in circulating tumor cells (CTCs), it is difficult to use AR expression as a predictive pre-treatment factor [9]. However, progress in next-generation sequencing methods may soon allow patient samples to be analyzed for characterization of AR-activating mutations or splice variants. This may also allow molecular selection of patients for the development of novel compounds. AR independent molecular pathways are also emerging as drug targets [10].

DLT Definition and Period

Assessments of DLT were developed in the context of cytotoxic drug development and therefore focused on hematological toxicity [11]. Consequently, DLTs are usually assessed in the first 4 weeks of treatment. However, in the development of targeted treatments, these DLT definitions may not be appropriate. An example is the early clinical development of the immune therapy ipilimumab in CRPC patients. Although no DLTs occurred within the first 5 weeks of commencing treatment, a substantial proportion of patients (32 %) experienced immune treatment-related grade 3 and 4 adverse events [12]. Additionally, standard DLT criteria may not capture cumulative toxicities that may significantly impact quality of life or treatment tolerability. Long-term or cumulative toxicity can be addressed by including an additional DLT criterion: "any other toxicity, at any time during trial treatment, that in the opinion of the investigators and medical monitors is dose limiting" [13].

Pharmacokinetic (PK) Dosing and Intrapatient Dose Escalation

Many Phase I trials recommend Phase II doses that are not well tolerated in non-trial populations, but selecting a widely tolerable dose risks underdosing many patients. This problem can be lessened using intrapatient dose escalation or PK-guided therapeutic dosing. Significant challenges may be encountered in defining a therapeutically active dose without causing severe side effects. In the Phase I clinical trial of abiraterone, nine-fold variations in the area under the curve (AUC) and maximum plasma concentration (Cmax) were reported [14]. Many oral targeted treatments have similar issues of significant interpatient PK variability. Furthermore, it is often not clear what PK markers to use. An established AUC value, indicating optimal balance between efficacy and toxicity, is often not known [15]. Additionally, tumor penetration can be

variable, further complicating the goal of achieving therapeutically active doses in tumors [16].

There are both biological and ethical rationales to consider intrapatient dose escalation in early phase clinical trials. Intrapatient dose escalation allows patients without significant toxicity increased potential for benefit, as long as there are no major safety concerns [17]. Apart from potential benefit for individual patients, intrapatient dose escalation may generate valuable information on "reversal of resistance" and may help to minimize the risk of protracted phase I trials [13], but also complicates the interpretation of treatment-related adverse events and DLTs. Other practical difficulties with PK-guided treatment include the logistics of repeat blood sampling to investigate patient exposure at each dose and difficulties achieving target AUC [18].

Safety Monitoring

Safety monitoring in early phase clinical trials aims to detect and minimize harmful effects of investigational agents. It underpins the ethical conduct of clinical research and is a key component of all clinical trial designs. Additional safety issues can arise in men with CRPC due to extensive bone involvement and older participant age, which can lead to increased bone marrow toxicity, as was observed in the clinical development of cabazitaxel [19]. However, in a cohort of 442 CRPC trial participants, mortality on or within 30 days of treatment was low and the majority of deaths were associated with progression of the underlying disease, suggesting that trial participation can be a safe option for CRPC patients [20].

Phase II Clinical Trials

Trial Design

Phase II studies are an important step in gauging the antitumor activity of new compounds. Explicitly stated clinical development intentions for compounds should allow the design of appropriate early phase trials with sound statistical plans. There are a number of designs for Phase II trials testing targeted therapies in CRPC. Both single arm and randomized trials are utilized, depending on the study aim and the availability of historical controls. The inclusion of biomarker based stratification in these trials appears to be a critically important concept, allowing researchers to identify target populations for Phase III testing.

In 2007 the international Methodology for the Development of Innovative Cancer Therapies task force published recommendations for the design and conduct of Phase II trials of targeted anticancer therapies, including: consideration of multiple endpoints and designs; incorporation of novel endpoints and designs; and comprehensive trial reporting including the publication of negative trials [21]. Nonetheless, ongoing controversies surround the extent of in vivo efficacy data required to support clinical drug development and the value of disease stability or functional imaging changes during early phase testing [22].

Early phase trials also provide an opportunity to incorporate translational research. In the example of the CYP-17 inhibitor abiraterone, the early phase clinical testing included ultra-sensitive endocrine assays that supported the mechanism of action [14] and CTCs as a novel measure of activity [23–25].

Trial Adaptations

Phase II oncology trials increasingly incorporate multiple stages or adaptive designs, with careful consideration of both clinical and statistical factors informing trial design [26, 27]. Multi-stage Phase II trials in CRPC have included simultaneous designs, allowing the testing of multiple schedules against a single control arm [28] and sequential designs, moving from a non-randomized to randomized design depending on early response data, as in the Phase II trial testing the PARP inhibitor olaparib (TO-PARP, clinicaltrials.gov identifier NCT01682772) [29]. TO-PARP is a novel adaptive, biomarker-driven

clinical trial, aiming to identify a molecularly characterized patient population with sensitivity to PARP inhibition. Multiple adaptations were built into the TO-PARP design to molecularly characterize a subgroup of patients sensitive to PARP inhibition and accelerate the development of olaparib in the CRPC population. The first stage included predictive biomarker identification and evaluation, followed by validation of the identified biomarker(s), leading potentially to definitive Phase III testing. Adaptations included pre-defined early stopping rules for futility or unprecedented efficacy, interim analyses, incorporation of early response biomarkers (CTCs, whole body diffusion-weighted magnetic resonance imaging (DW-MRI)), and seamless transition into a randomized Phase III clinical trial.

Both European and American regulatory authorities have encouraged adaptive clinical trial designs [30, 31], aiming to reduce the costs associated with drug failure in late stage development. Widely cited examples of adaptive clinical trials include the BATTLE trial in non-small cell lung cancer and the I-SPY2 study in locally advanced breast cancer [32, 33].

The primary risk of adaptive processes is the potential for increased type I errors, whereby trials produce a false positive result or are difficult to interpret due to the small sample size and multiple variables evaluated. These errors can be controlled by pre-specifying all adaptive measures. Type II errors, in which a true treatment effect is not observed, may also occur because of selection processes, suboptimal adaptive measures, or lack of statistical power and small sample size [34].

Utility of Phase II Trials

Phase II trials with positive signals of activity do not always result in positive Phase III trials [35], due to the use of endpoints lacking clinical relevance, changes in post-trial treatments, the study of different patient populations, and differences in disease and patient characteristics in larger, more "real-world" trial populations. However, in the example of docetaxel-combination studies in CRPC, none of the eight published negative Phase III studies could be regarded as having clear Phase II activity signals for the combination approach [36]. Extrapolation from single-agent studies proved unsatisfactory, as did enthusiasm based on the achievement of secondary study endpoints. It would seem that the completion and interpretation of a well-designed Phase II trial remains currently one of the biggest hurdles in prostate cancer research.

Furthermore, critically, the activity reported in a Phase II clinical trial must be interpreted with caution, because of the large confidence intervals (CIs) associated with small cohort sizes. For example, a response rate of 40 % is often associated with a 95 % CI of 20–60 %. Activity CIs in randomized Phase II clinical trials may therefore overlap and consequently do not allow adequate comparison of the two arms [37]. A striking example of this was the purportedly (but false) positive Phase II trial of iniparib with chemotherapy in metastatic breast cancer [38], leading to a large and costly negative Phase III trial [39].

Defining Trial Populations

Standard criteria defining oncology trial populations require modification for the CRPC setting. Appropriate populations are usually defined using clinical and biochemical markers of physiologic fitness, rather than age. Some of these measures, however, are influenced by age and can indirectly discriminate against older patient participation. One such example is the Cockcroft–Gault tool for estimating creatinine clearance [40], which includes a significant adjustment for subject age and can result in a low estimation even in the face of a low-normal creatinine.

Criteria to define cancer progression for patients with CRPC can also be both complex and cumbersome. Men with prostate cancer commonly have bone-predominant disease, which is not monitored effectively on standard computed tomography (CT) or bone scan. Criteria for prostate-specific antigen (PSA) progression in trials of men with CRPC have been well defined by the Prostate Cancer Working Group (PCWG) [41] and allow most men, even low PSA secretors, the

opportunity to participate in clinical trials. However, PSA criteria have less relevance in selecting populations with the appropriate progression for testing non-AR targeting therapies and PSA progression does not always accompany clinical or radiological progression [42].

Patients participating in Phase I studies often have advanced disease and have exhausted standard treatment options. Such patients can deteriorate quickly, compromising safety boundaries and complicating the assessment of drug safety. Prognostic scores have further attempted to identify patients unlikely to benefit due to poor life expectancy, or at particular risk of toxicity [43, 44]. The Royal Marsden score has been validated in a large population of patients participating in trials of targeted therapy [45] and performed well with addition of CTC counts [46]. The ability to clearly identify the target in CTCs may also support patient selection for trial entry and evaluate intrapatient diversity and clonal evolution. Such selection based on predictive biomarkers at study entry can offer the potential for improved efficacy, but may also slow trial recruitment. Nevertheless, collection of CTCs offers the opportunity both for quantification and the evaluation of antitumor activity [9, 23]. Since multiple distinct genomic subtypes of CRPC have been described, resulting in molecularly diverse cancers, such an approach may be a key to future successful drug development. ETS gene fusions and PTEN loss are well described in CRPC [47, 48], as is the increasing prevalence of neuroendocrine differentiation [49]. As yet, these subpopulations have not been stratified within clinical trials, but heterogeneity within tumors and between tumors within individual patients may pose significant challenges in this respect.

Defining Trial Endpoints

Post-treatment CTC counts are being evaluated as surrogate endpoints for overall survival in patients with CRPC. With the rapid pace of successful drug development in CRPC the need for biomarkers that can act as markers of surrogacy for the registration and approval of new compounds has also become a key priority [50].

Predictive Biomarkers

Co-development of biomarkers for targeted treatments may increase the success rate of drug registration. However, currently no predictive markers have been identified in CRPC. The BRAF mutation in melanoma and the EML–ALK translocation in lung cancer are good examples of co-development, allowing identification of subgroups of patients most likely to benefit from targeted treatment. As described in the pharmacologic audit trail (see Fig. 13.1), Phase I/II clinical trials can include the exploration and development of biomarkers [51]. Based on robust biological hypotheses, multiple circulating and tissue-based biomarkers can be interrogated for evidence of target modulation or inhibition, to determine a patient population that might benefit from the targeted treatment. The TO-PARP trial provides a framework for the co-development of drug and companion diagnostics in a Phase II setting, with identification (see Fig. 13.2; Part A) and subsequent qualification of a potential predictive biomarker suite (Part B).

Response Endpoints

Limitations in assessing response in CRPC have long been recognized. Neither PSA nor derived measures such as PSA change and PSA velocity have met surrogacy criteria for survival benefit [50]. Despite this, PSA remains a common measure of treatment response [41]. PSA is widely used in Phase I/II trials, although the mechanism of action of the therapy may render PSA an unhelpful marker of activity. For example, immunotherapies may demonstrate improved survival without impacting PSA kinetics [52, 53].

In the 1970s bone scintigraphy was adopted for the detection of bone metastases [54]. False negative scans due to lack of osteoblastic response or small lesion size, lack of specificity, and difficulties with reproducibility were quickly recognized [55]. Despite these issues, bone scans became part of the standard staging for men with prostate cancer. The PCWG2 recommended assessing progression based on the appearance of new lesions, rather than more subjective measures relating to tracer intensity,

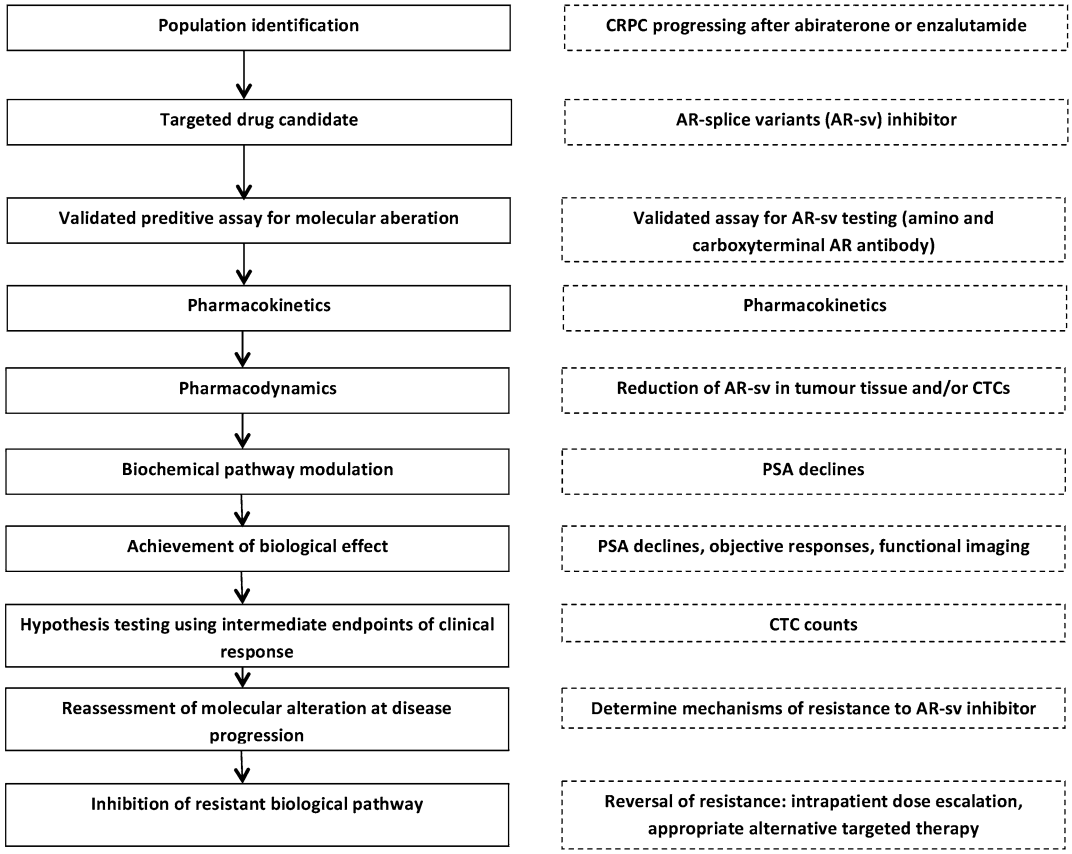

Fig. 13.1 A pharmacologic audit trail for clinical trial design. *Solid boxes* contain the conceptualized trial framework; *dashed boxes* demonstrate the example of a new therapy targeting androgen receptor (AR) splice variants (AR-sv) in castration-resistant prostate cancer (CRPC). CTC: circulating tumor cell; PSA: prostate-specific antigen

or individual lesion size or area [41]. More recent attempts to improve bone scan assessment have included computer-aided interpretation, which showed a good ability to reproduce the bone lesion area, intensity, and count assessments of a highly experience nuclear medicine physician [56].

The Response Evaluation Criteria in Solid Tumours (RECIST) were published in 2000 and aimed to standardize response assessment for soft tissue disease manifestations [57]. These criteria were incorporated in a modified form in the PCWG2 recommendations, including 3-monthly restaging with CT and bone scans. Alternative imaging modalities have been proposed and although the optimal modality and technique remains unclear, there is increasing enthusiasm for replacing the traditional methods of prostate cancer assessment. Whole-body DW-MRI has been shown to outperform Tc 99 m bone scanning and may be suitable to assess treatment responses [58]. 18 F-Choline positron emission tomography-CT (PET-CT) may also offer improved sensitivity and specificity in detection of prostate cancer metastases [59]. However more data are required to bring these imaging modalities into routine clinical practice and trial design.

Novel biomarkers for efficacy monitoring include CTC counts and biology-driven markers of treatment effect [50]. These strategies require a rigorous process to prove surrogacy, but can be useful to identify patients likely to benefit from targeted therapy. Encouraging results were

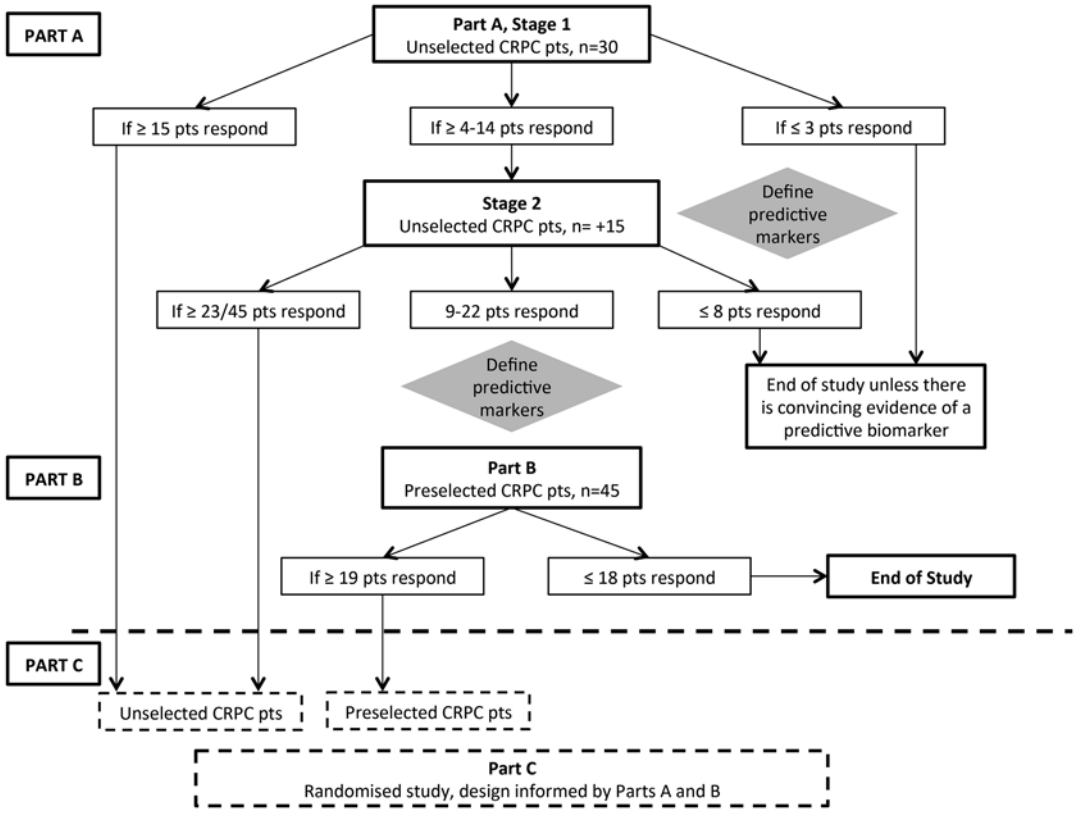

Fig. 13.2 The Phase II TO-PARP trial framework. CRPC: castration-resistant prostate cancer; pts: patients; n: number

observed in the COU-AA-301 trial, where CTCs in combination with LDH demonstrated survival surrogacy on an individual patient level [60, 61].

Conclusion

The past decade has witnessed strong interest in prostate cancer research, resulting in six survival-prolonging treatments for men with CRPC and a deeper understanding of the complex molecular drivers of progression. Molecular pathways of growth are being targeted using novel agents and combinations. Successful drug development requires a comprehensive strategy, from intelligent early phase trial design, through to appropriately designed and powered Phase III trials. Multiple valid designs and endpoints can be utilized, but selecting the best fit for the investigational target will maximize the likelihood of successful drug registration. Incorporation of translational science during early phase development can benefit both in the later stage trial design and in increasing our understanding of the underlying biology of CRPC.

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Defining Clinical Endpoints in Castration-Resistant Prostate Cancer

14

Jorge A. Garcia and Robert Dreicer

Introduction

Although this is an exciting time for prostate cancer (PCa) therapeutics, drug development in men with castration-resistant prostate cancer (CRPC) faces significant challenges. Disease biology and the well-recognized heterogeneity of prostate cancer are some of the inherent disease-specific issues that continue to affect trial design [1]. Similarly, traditional endpoints used in early phase I and II studies do not appear to be useful when evaluating survival outcome in phase III trials. Adding complexity to a challenging area, some newer PCa trials design guidelines do not align with current US Food and Drug Administration (FDA) requirements necessary for drug registration and approval [2]. To date, there are no validated surrogate endpoints capable of predicting benefit of early treatment in men with metastatic castration-resistant prostate cancer (mCRPC). Historical endpoints such as prostate-specific antigen (PSA), Response Evaluation Criteria in Solid Tumors (RECIST), and progression-free survival (PFS) have failed to reliably predict survival benefit in this patient population [3]. Although clinically relevant, assessments of QOL and pain have not been reliable trial endpoints. Additionally, changes in the ubiquitous biomarker PSA, an androgen receptor (AR) gene product may be relatively uninformative when used in the setting of non-AR directed therapy and in general has not been found to correlate with more accepted outcome measures such as survival [4].

The evolving complexities of the design and conduct of a clinical trials, FDA requirements for registration, and the routine clinical use of a variety of endpoints are now increasingly challenged by the rapidly evolving understanding of the biology of CRPC and the importance of the AR as an therapeutic target for drug development [5, 6]. Adding to the challenge is the widespread availability of the AR targeting agents enzalutamide and abiraterone acetate (AA) without a prospectively tested and validated understanding of their optimal use in terms of sequence and patient selection [7–10].

Another pressing question is how to incorporate non-AR targeting therapeutics such as immunotherapy into routine clinical practice as these agents by virtue of their mechanism of action may require "different" endpoints, especially if they are used early in the disease course. Sipuleucel-T is a perfect example of the apparent disconnect between traditional endpoints such as prostate-specific antigen (PSA) response and objective response rate (ORR) and overall survival [11].

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F. Saad and M.A. Eisenberger (eds.), *Management of Castration Resistant Prostate Cancer*, Current Clinical Urology, DOI 10.1007/978-1-4939-1176-9_14, © Springer Science+Business Media New York 2014

Defining rational and practical endpoints that can be used in CRPC trial design is desperately needed, especially as clinical oncology is rapidly, albeit uncertainly, moving towards an era of genomics and precision medicine while at the same time shifting rapidly to an era of cost-containment making new drug development that much more challenging. In this chapter we review traditional endpoints that have historically been used in CRPC trial design. We also discuss potential new strategies that could be incorporated in future CRPC studies.

Understanding Castration-Resistant Prostate Cancer (CRPC)

Although from the perspective of managing an individual patient, CRPC remains a very heterogeneous disease, advances in the biology of CRPC have codified the importance of AR pathway and its role in the pathogenesis and progression to a castration-resistant state [5, 12]. Among the challenges when attempting to define outcomes in men with prostate cancer has been the lack of validated surrogate markers that can be used to predict overall survival. Disease heterogeneity also plays a role in the evolution to castration-resistant disease given the likelihood that multiple pathways in AR resistance are involved and with the clinical definition of castration-resistant disease somewhat empirically defined as disease progression be it biochemical, i.e. PSA-only or with overt radiographic and/or symptomatic progression in the setting of a "castrate level" (<50 ng/dl) of testosterone [12]. As such some men can present with serologic progression-only while others develop symptomatic disease. Similarly, many others are detected by "routine" imaging studies. More recently the importance of disease related symptoms when selecting CRPC patients for clinical trials has been increasingly considered and included into study designs. Differences among these groups of patients, i.e. asymptomatic versus minimally symptomatic versus symptomatic, have become increasingly relevant when interpreting trial results as more contemporary studies have included a placebo component and utilize pain and quality of life (QOL) instruments in their trial design [13, 14]. With the availability of Sipuleucel-T, abiraterone acetate (AA) and enzalutamide, the number of previous therapies, treatment sequence, and the possibility of early introduction of chemotherapy will likely impact the selection of endpoints for future clinical trials.

Additional challenges include the incorporation of serum and tissue biomarkers in mCRPC trial designs. In this evolving era of precision medicine where we are faced with understanding the role and the potential utility of next generation genomics, well-defined strategies that integrate biologically relevant markers will be needed to facilitate progress towards a more rational approach to the management of men with CRPC [15, 16].

Traditional Endpoints in CRPC Trials

PSA Response

Since its introduction in the late 1980s PSA has been the most commonly used biomarker to both evaluating treatment efficacy and predicting prognosis for patients with advanced PCa [17, 18]. While undetectable PSA values are often achieved shortly after primary, curative intent therapy with either surgery or radiotherapy, absolute PSA values, percentage of PSA decline, and other methods of assessing PSA kinetics are more difficult to interpret in the advance disease setting [19, 20]. Assay variability and the capriciousness of PSA assessments outside the context of trial requirements have complicated the interpretation of clinical studies as many physicians rely on PSA values when making treatment decisions in their routine clinical practice. This impact may be felt to a greater extent when management changes are considered for men based on PSA progression when receiving therapeutic agents with non-AR-dependent mechanisms of action. The relationship between objective response and PSA changes over time is also challenged by the common disconnect between PSA decline and tumor burden reduction often observed in men receiving therapy for mCRPC. Historically PSA response and time to

PSA progression have been incorporated in prostate cancer clinical trials [21]. Retrospective studies conducted in the late 1980s suggested that PSA declines could be interpreted as a good indicator of treatment response [22, 23]. Investigators at the University of Michigan provided preliminary evidence that men who failed to achieve a PSA decline >50 % while receiving chemotherapy doubled their risk of death compared to those able to achieve that level of biochemical response [23]. Another example of the challenges of using PSA response as an endpoint was illustrated by a translational study of suramin, a highly charged polysulfonated naphthylurea capable of binding a number of proteins, including a variety of growth factors such as basic fibroblast growth factor. Suramin inhibited PSA secretion with no cytotoxic effect and there was no association between the percentage of PSA decline (<50 versus ≥50–75 % versus ≥75 %) and survival [24]. The results of this analysis conflicted with the results of their phase III study that evaluated Suramin versus hydrocortisone in men with mCRPC where a post-treatment PSA decline of ≥50 % lasting ≥4 weeks was associated with longer PSA and overall survival. This difference was maintained after adjusting for burden of disease and baseline PSA value [25].

The uncertain utility of PSA as a response parameter and inability to define standard endpoints for trials in advanced prostate cancer led a number of academic investigators involved in new drug development in prostate cancer to come together and release guideline statements from the Prostate-Specific Antigen Working Group. These guidelines focused on men with CRPC and defined eligibility, outcome measures and standardized the use of PSA in the context of clinical trials [26].

A number of trials studies evaluating docetaxel-based chemotherapy analyzed PSA response as a potential surrogate marker for survival [18, 27]. In another clinical setting a large US Intergroup study evaluated the role of continuous versus intermittent androgen deprivation therapy (ADT) in men with de-novo metastatic prostate cancer exploring the impact of the degree of PSA nadir on overall survival following 7 months of ADT [28, 29]. In that study while the median overall survival for men with nadir PSA values <0.2 ng/mL at 7 months of induction ADT was 75 months, patients with PSA nadir of 0.2–4.0 and >4 mg/mL had a median survivals of 44 and 13 months, respectively ($p < 0.001$). These results support the utility of nadir PSA as a potential surrogate endpoint in this clinical setting. Although nadir PSA is not an endpoint commonly employed in the castration-resistant setting, the association between PSA decline and outcome has been analyzed in contemporary trials. Secondary PSA analysis of TAX 327, a randomized phase III trial evaluating docetaxel plus prednisone given either every 3 weeks or weekly versus Mitoxantrone plus prednisone given every 3 weeks demonstrated a 60 % risk reduction of death in mCRPC patients achieving PSA declines greater than 50 % compared to their baseline PSA value [30]. In contrast, SWOG 9916, a similar randomized phase III study evaluating docetaxel-based chemotherapy in mCRPC examined the impact of the percentage of PSA decline on median overall survival. To determine whether PSA decline could be utilized as a surrogate marker for outcome, a Cox model testing for significance between treatment and survival time after adjustment for PSA decline was developed. PSA declines of 10, 20, 30, 40, 60, 75, 80, and 90 % were considered. After adjusting for a post-treatment PSA decline of 30 %, the treatment effect was no longer significant. While these results suggested that a PSA decline ≥30 % during treatment with docetaxel-based chemotherapy could be used as a surrogate marker for survival, PSA decline by itself could not fully explain total dependence between treatment received and the observed survival outcome [31].

While the utility of PSA response remains undefined, it is a rationale target in the context of AR directed therapeutics. Agents such radium-223 and Sipuleucel-T and a number of novel agents in development however do not appear to block AR transcription and thus the lack of impact on PSA decline. In studies of these agents, PSA is a far less attractive biomarker for activity. Taking this and other response questions into consideration the role of PSA, PSA-related measures, and other outcome measures for trials in mCRPC were reconsidered by the Prostate Cancer

Clinical Trials Working Group (PCWG2) [32]. Recommendations from this group included an increased emphasis on time-to-event endpoints (i.e., failure to progress) when moving from phase II to III trials. Likewise, PCWG2 recommended that PSA should not be used to define treatment efficacy nor as a criteria for drug discontinuation in individual patients or studies. Contrary to the recommendations from PCWG1 regarding the reporting of PSA response rates, PCWG2 recommended against such actions as serologic responses appears to have little value given the uncertain significance of a defined degree of decline from baseline and the absence of prospective evidence of its utility as a surrogate of clinical benefit. PCWG2 did however recommend that in the design of mCRPC studies, the percentage of change in PSA from baseline to 12 weeks (including those who discontinue therapy earlier), as well as the maximum decline in PSA that occurs at any point after treatment be reported for each patient in the form of a waterfall plot [32]. These recommendations are now widely accepted and have been incorporated in the design of modern trials evaluating agents such as cabazitaxel, Sipuleucel-T, abiraterone acetate (AA), radium-223, and enzalutamide [7, 9, 11, 33].

Recently, Halabi and colleagues analyzed PSA data from the phase III study evaluating cabazitaxel versus mitoxantrone and prednisone in mCRPC patients previously treated with a docetaxel-containing regimen [34]. The primary aim of their analysis was to confirm previous findings from SWOG 9916 about the potential surrogacy of a PSA decline (≥30 %) within 12 weeks of treatment initiation with docetaxel-based chemotherapy. When Prentice operational criteria were applied, the treatment received was a statistically significant predictor for overall survival [35]. Similarly, a PSA decline of >30 % was also a significant predictor for overall survival with a hazard ratio of 0.52 ($p<0.001$). Unfortunately, in the multivariable model both PSA decline and individual treatment arm remained statistically significant thus failing to meet criteria for surrogacy. When using Prentice criteria, a marker is considered a surrogate endpoint if it is statistically significantly associated ($p<0.05$) with overall survival in both univariate models. However, in the multivariable model, the marker but not treatment arm needs to be statistically significant. Furthermore, the proportion of treatment effect explained for PSA decline of ≥30 and 50 % in this report did not exceed 0.50, the lower bound of the 95 % CI, suggesting a lack of surrogacy [34].

Significant PSA responses were also observed in the mCRPC trials with the CYP-17 inhibitor abiraterone acetate. While in the post-docetaxel study a PSA response was observed in 29.5 % of patients, almost 62 % of patients in the chemotherapy-naive study achieved a PSA decline of ≥50 % [8, 9]. Similarly, the PSA responses observed in the post-chemotherapy enzalutamide phase III trial and the enzalutamide chemotherapy-naive phase III study were 54 and 78 %, respectively [7, 36]. Despite the impressive serologic responses observed in these studies, the utility PSA decline as a surrogate endpoint remains undefined.

Limitations of PSA Endpoints

As we have described, PSA response has failed to meet requirements for surrogacy for survival in trials evaluating cytotoxic therapy, yet PSA testing remains a critical parameter for practicing physicians and their patients. While PSA values in the individual patient is almost certainly ordered too often and frequently used to make therapeutic decisions in routine clinical practice irrespective of the clinical setting, it is this routine and uncontrolled practice that has affected the integrity and outcome of many US-based prostate cancer studies. Although PSA declines in patients receiving chemotherapy are highly prognostic and perhaps an easy measure to evaluate response within a few months of initial treatment, there is no cut point that can be used to fully predict benefit for an individual patient. Similarly, transient elevations of PSA while receiving novel agents could be the result of a "flare phenomenon" and not representative of true disease progression [32]. Early recognition of this phenomenon is critical to avoid early drug discontinuation in routine practice and clinical trials.

Whether PSA can be used as a biomarker for assessing anti-tumor activity and disease outcome when evaluating novel non-AR targeting agents remains unknown. Two recent, large, randomized phase III studies evaluating active agents in mCRPC highlight the disconnect that exists between PSA and overall survival. The IMPACT trial (Immunotherapy for Prostate Adenocarcinoma Treatment), a phase III, double-blind, placebo-controlled study enrolled 512 men with asymptomatic or minimally symptomatic mCRPC randomizing them 2:1 to receive sipuleucel-T or placebo [11]. Treatment with sipuleucel-T prolonged median overall survival, the primary endpoint, by 4.1 months compared to placebo (25.8 versus 21.7 months, respectively) and reduced the risk of death from any cause by 22.5 % ($p=0.032$). The significant improvement in overall survival in men with symptomatic or minimally symptomatic metastatic CRPC in men receiving sipuleucel-T was obtained without evidence of a measurable anti-tumor effect such as objective response in soft tissue or PSA reduction of at least 50 %. These results have added to the controversy regarding the role and utility of this novel immunotherapy in mCRPC [37].

More recently the alpha-emitter, radium-223 demonstrated an overall survival improvement in men with mCRPC with predominant bone metastases [33]. In addition to the statistically and clinical relevant survival benefit radium-223 delayed the development of symptomatic skeletal events (SSEs). Of interest however is that these clinically meaningful endpoints were obtained with minimal impact on PSA. Although not surprising based on its presumed mechanism of action, the lack of association between PSA decline and survival provides additional evidence of the need to move away from PSA endpoints in this patient population. Although PSA progression defined by either PCWG1 or 2 has been removed from contemporary CRPC trial designs, treatment discontinuation secondary to rising PSA while on therapy remains a major issue in routine clinical practice. Although retrospective analyses of both docetaxel trials (TAX327 and SWG9916) suggested that an increase in PSA at 3 months of therapy correlates with poor overall survival, to date the utility of PSA progression in the castration-resistant setting has not been prospectively validated and remains undefined.

Objective Response

Tumor burden reduction has been the most traditional method used to assess efficacy in cancer therapeutics. Phase II studies in many solid tumors assess the effect and duration of a treatment using response rates defined by Response Evaluation Criteria in Solid Tumors (RECIST) [38]. Although no prostate cancer therapeutic has been FDA approved solely on this basis, objective response assessment is typically included in phase II mCRPC trial designs. With the exception of sipuleucel-T and radium-223, all of the agents FDA approved for mCRPC demonstrated a degree of objective response in phase III testing. Given the bone tropism of advanced PCa and the lack of objective means to assess response in bone the utility of objective response as a means of assessing therapeutic benefit in mCRPC has been somewhat limited. In most trials conducted in the current era, only 35–40 % of patients with mCRPC have overt evidence of lymph node metastases while less than 5–8 % of patients present with visceral disease [39].

Following the results of the two major phase III trials of docetaxel, efforts to interrogate the potential for ORRs to provide some degree of surrogacy for survival have been explored. In the TAX 327 trial, patients randomized to every 3 weeks docetaxel arm demonstrated an ORR of 12 % while those receiving docetaxel plus estramustine in SWOG 9916 manifested a 17 % response rate [30, 31]. A subsequent report by the TAX327 investigators analyzed the association of measurable tumor response with survival. Tumor response was evaluated by World Health Organization (WHO) criteria, for which the product of the largest diameter and its perpendicular is summed for predefined lesions.

Of the 1,006 patients enrolled on the TAX 327 study, 412 (41 %) had measurable disease. Of those, 37 patients exhibited an objective response (CR/PR, 9.0 %). Partial responders demonstrated

longer median overall survival (29.0 months) than patients with stable or progressive disease, with these findings remaining after evaluation with a landmark analysis [40]. While all patients with measurable tumors who achieved an objective response exhibited PSA declines, only a proportion of patients with PSA declines exhibited objective responses. Although this data suggests that an objective response by WHO criteria in men receiving docetaxel for mCRPC could translate into a survival benefit, the clinical relevance of this finding in the setting of widely metastatic bone disease remains unclear.

Recently developed AR targeted agents have demonstrated objective responses in those patients with measurable disease. When evaluating the response rate of abiraterone plus prednisone in men with measurable disease participating in the post-docetaxel abiraterone phase III trial, 14 % of patients in the abiraterone arm compared to 3 % of patients in the prednisone plus placebo arm achieved a RECIST-defined PR or CR ($p<0.001$) [8]. In the chemotherapy-naïve trial, patients receiving abiraterone plus prednisone demonstrated an ORR of 36 % compared to 16 % of patients receiving prednisone/placebo ($p<0.001$) [9]. It is important to recognize that in contrast to the older docetaxel-based chemotherapy studies, the abiraterone phase III trials utilized RECIST criteria which incorporates a different criteria for nodal disease response assessment [38]. Perhaps the greatest number of patients with measurable disease ever enrolled in an mCRPC trial was the post-docetaxel trial of enzalutamide, a randomized, double-blind, placebo-controlled study which randomized patients with mCRPC who had previously been treated with one or two chemotherapy regimens to receive enzalutamide or placebo. In this trial over 92 % of patients receiving enzalutamide had bone metastases, among them almost 40 % had extensive disease (>20 bone lesions). Soft tissue disease was evaluable in over 70 % of patients. Lymph node metastasis was present in approximately 55 % of patients while visceral liver and lung disease was seen in 11 and 15 % of patients, respectively. The ORR reported was 29 versus 4 % in favor or enzalutamide therapy ($p<0.001$) [7]. Recent results from the chemotherapy naïve phase III trial of enzalutamide (PREVAIL) reported a response rate of 58.8 % in patients treated with enzalutamide in contrast to a. 4.9 % in the placebo control arm ($p<0.0001$). Of interest is that 20 % of patients achieved a RECIST-defined CR [36]. Both studies evaluating enzalutamide (pre and post-chemotherapy) used RECIST 1.1 to measure tumor response. Some of the important changes made in RECIST 1.1 criteria include: (a) reduction in the number of lesions to be assessed (from a maximum of ten to a maximum of five total; (b) number of lesions per organ also down to two, maximum); (c) lymph nodes are now considered measured lesions if larger than 1.5 cm in short axis; (d) confirmation of response is required for trials with ORRs as the primary endpoint but not in randomized studies since the control arm serves as appropriate means of interpretation of data; (e) in addition to the 20 % standard increase for progressive disease, a 5 mm absolute increase is also mandated [41].

Several issues constrain the use of objective response as primary endpoint in mCRPC trials. The major issue is of course the bone tropism of the disease and the current inability to objectively and reproducibly assess changes in bone lesions. Historically, bone metastases have been evaluated by bone scintigraphy using technetium 99 m-labeled methylene diphosphonate (MDP). Interpretation of bone scintigraphy is operator dependent and the significance of changes in size or uptake in existing lesions lacks prospective clinical correlation. Additionally, scintigraphy does not directly reflect prostate cancer cellular activity as it reveals the incorporation of MDP into hydroxyapatite, a major component of the bone compartment. Moreover, irrespective of the therapeutic agent, complete resolution of bone disease is exceedingly uncommon. Recently a randomized phase II trial with a discontinuation design demonstrated complete resolution of bone scintigraphy findings in a series of men with mCRPC and bone metastases receiving cabozantinib, an oral dual C-MET and VEGF-R2 inhibitor [42]. This trial employed standard bone scintigraphy for the evaluation of bone metastases. Although it is unclear if bone scintigraphy is

the best imaging methodology when using this novel agent, ongoing phase III trials are further evaluating the clinical activity and safety of this compound in the castration-resistant setting and should serve as a proof-of-principle to determine if complete eradication of bone disease is indeed possible in advanced PCa.

Newer imaging techniques such as 18F-flouride PET/CT or ^{11}C choline PET may improve our ability to detect a treatment effect in the bone compartment [43, 44]. However, these imaging modalities are costly, operator dependent, and are not yet a part of routine assessment of the disease in either clinical trials or routine clinical practice. At present, when bone scintigraphy is the sole indicator of progression, PCWG2 defines progressive disease in bone when at least two or more new lesions are seen on bone scintigraphy compared with a prior scan for trial entry and these are confirmed on a subsequent set of images. In an attempt to lessen the impact of reader variability, PCWG2 advocates that any change in tumor size at the first 12-week assessment be confirmed in a subsequent scan. Likewise, changes in bone scans should be confirmed with repeat imaging to avoid the false positive impact of flare reactions or unreported trauma. As with changes in PSA, PCWG2 suggests that changes in the size of target lesions be reported as a waterfall plot to facilitate comparison between studies [32]. It is unclear if responses observed in soft tissue disease parallel improvement of bone disease. Also not known is the impact of systemic therapy in local disease when the prostate gland remains in place. How to optimally interpret changes in tumor burden reduction in the setting of stable bone scintigraphy remains undefined and contributes to the limitations in the use of objective response in clinical trial design.

Although further exploration of the potential association between response rate and survival in patients treated with next generation AR targeted agents is ongoing, the major clinical use of this assessment will be for prognostic purposes and in the early assessment of efficacy among men with measurable disease. Together with PSA changes and patient-reported outcomes, objective response could be a clinically useful tool in the routine assessment of treatment benefit over time.

Time-to-Event Endpoints

Delaying or preventing disease progression in patients with CRPC has become an attractive endpoint for registration trials. Admittedly the design and conduct of trials in the non-metastatic CRPC setting is far more challenging as this often requires patients to remain on therapy until detectable metastases occur. This is especially difficult when the agent in question does not have a direct impact on PSA levels. Even if one is able to delay metastases-free survival, the association between this endpoint and overall survival remains undefined. Studies evaluating the activity of the novel endothelin A inhibitor atrasentan provide perspective on the challenges when time-to-event endpoints are used to design trials in non-metastatic CRPC patients. A large phase III trial randomized 941 patients with rising PSA in the castrate setting in patients with no evidence of metastatic disease to either atrasentan or placebo with time to disease progression, defined as development of metastatic disease, as the primary end point [45]. No difference in the time to progression in patients receiving atrasentan; ($p = 0.288$) was observed. Similarly, no effect on overall survival, a secondary endpoint of the trial was noted. In addition to the lack of efficacy, significantly more patients receiving atrasentan discontinued the trial prematurely. Although treatment discontinuation due to adverse events was common, a large number of patients discontinued therapy prior to reaching objective progression, the primary endpoint of the study, likely due to concern by both patients and physicians for PSA progression.

More recently, denosumab, a fully human anti-RANKL monoclonal antibody was evaluated for prevention of bone metastasis or death in non-metastatic CRPC patients. More than 1,400 patients were enrolled in this international study which demonstrated that denosumab significantly increased bone-metastasis-free survival by a median of 4.2 months compared with placebo ($p = 0.028$) [46]. Denosumab also significantly delayed time to first bone metastasis ($p = 0.032$). Despite this apparent benefit, there was no difference in overall survival between groups ($p = 0.91$).

Although targeting the bone microenvironment might be of clinical benefit to some patients, the lack of survival benefit observed in these trials has halted further development of purely bone targeted agents in the non-metastatic setting.

The evaluation of these agents in men with metastatic disease is somewhat different as skeletal-related events (SREs) have for some time been accepted endpoints for clinical trial design in CRPC. Despite failing to demonstrate a survival benefit, two agents, zoledronic acid and denosumab received FDA approval on the basis of skeletal-related event (SRE) prevention [47, 48]. SREs defined by the development of spinal cord compression, pathologic fracture, orthopedic surgery, or need for palliative radiation therapy have also been incorporated in the trials that led to the FDA approval of enzalutamide, abiraterone acetate, and the radiopharmaceutical radium-223. Especially relevant as we move into a new era in health economics is the role of zoledronic acid and denosumab in settings in which patients are receiving agents such as enzalutamide, abiraterone, and radium-223, agents that in addition to providing improvement in survival have also demonstrated a significant impact on delaying SREs or in the case of radium-223 the more clinically relevant SSEs [33, 49, 50]. PFS, defined as the time from study entry to disease progression in bone or soft tissue, symptoms, or death is a common solid tumor endpoint employed in phase II clinical trials. This parameter is increasingly of importance in mCRPC trials because this endpoint, in contrast to overall survival, is not confounded by subsequent therapy. Statistically PFS is an attractive endpoint because it can reduce the sample size of clinical trial and has the advantage that a comparable endpoint used in phase II can also be utilized in a subsequent phase III comparative trial reducing the risk of non-active agents moving to large definitive randomized phase III study [51]. Use of PFS in mCRPC has significant limitations including variability of the definition used for progression, measurement error, observer bias, assessment schedule, and missing or incomplete data [52]. More recently a composite endpoint of radiographic progression-free survival (rPFS) has been incorporated into CRPC trial design as it includes relevant clinical features that impact patient outcome. These have been defined by PWGC2 as objective progression, SREs, symptomatic progression, or death from any cause. Although followed during treatment, PSA progression is no longer a component of PFS and its use in trial design and routine clinical practice should be discouraged. While rPFS is now widely employed an endpoint for trial design in the chemotherapy-naïve setting, overall survival in the post-chemotherapy setting remains the gold standard for FDA approval. PFS has not been validated as an accurate indicator of survival and to date it has not in isolation been accepted as a regulatory endpoint. A post-chemotherapy phase III randomized placebo study evaluating the oral platinum analog satraplatin provides a case in point [53]. In this study, a composite endpoint of PFS and OS was employed. The median PFS was 11.1 weeks in the satraplatin arm and 9.7 weeks in the placebo arm. Cox proportional hazards models revealed a significant 33 % reduction in risk of progression or death favoring satraplatin versus placebo ($p=0.001$). Despite achieving its composite primary endpoint of PFS, the study failed to demonstrate a survival benefit. The median survival for the stratified intent to treat analysis was 61.3 weeks for satraplatin and 61.4 weeks for placebo (HR 0.98; $p=0.80$).

Although PFS has been used as an endpoint in contemporary studies including cabazitaxel, abiraterone, and enzalutamide in chemotherapy-treated mCRPC patients, overall survival was the primary endpoint. Independently, each of these studies has demonstrated that in the post-chemotherapy setting that overall survival was a reasonable gold standard endpoint for drug approval. The median survival in the cabazitaxel phase III trial was 15.1 months in the cabazitaxel group and 12.7 months in the mitoxantrone group demonstrating a 30 % reduction in death in men receiving cabazitaxel ($p<0.0001$) [54]. Similar survival findings were reported in the recently updated analysis of the post-chemotherapy abiraterone trial. At a median follow-up of 20.2 months median survival for the abiraterone group was longer than in the placebo group 15.8 versus 11.2 months (HR 0.74; $p<0.0001$) [55]. The evaluation of enzalutamide in the same setting

led to similar findings with a median OS of 18.4 versus 13.6 months in the placebo group (HR for death in the enzalutamide group, 0.63; $p<0.001$) [7].

Contrary to previous attempts in this clinical setting, these three clinical trials also demonstrated the ability to statistically improve their secondary endpoint of PFS. Median PFS was 2.8 months in the cabazitaxel group compared to 1.4 months in the mitoxantrone group (HR 0.74; $p<0.0001$). Median rPFS 5.6 months in the abiraterone arm and 3.6 months in the placebo arm (HR 0.66; $p<0.0001$) and median rPFS of 8.3 versus 2.9 months; HR 0.40; $p<0.001$ in favor of treatment with enzalutamide. Of importance is the fact that PSA reduction, RECIST-defined response rates, and other QOL endpoints were also statistically superior in favor of each of these treatments. To date, no data exist concerning the potential surrogacy of PFS in the context of these clinical trials.

Despite the recent success of large number of trials in demonstrating a survival benefit in the post-docetaxel setting, the recent results from a phase III trial of orteronel a lyase inhibitor with similar properties to abiraterone represent a cautionary tale. In this large international study, with a design essentially identical to the post-docetaxel abiraterone trial, overall survival in the orteronel + prednisone arm was 17.0 months compared to 15.2 months in the prednisone + placebo group (HR 0.886; $p=0.1897$). Of interest in a prespecified analysis of survival in about 1/3 of patients treated in regions of the world in which at the time of the conduct of the study there was limited or no access to abiraterone and enzalutamide a survival benefit was observed [56]. Whether the impact of multiple active agents available to patients upon disease progression in studies of novel agents will negate the viability of OS as a meaningful endpoint is unknown, but of significant concern.

Patient-Reported Outcome Endpoints

Health-related quality of life (HRQoL) which incorporates issues related to both disease related symptoms and therapy related toxicity are clinically relevant considerations in the management of patients with mCRPC. Among these, disease related pain, assessed using a number of validated instruments and by assessment of opioid requirements has historically provided evidence felt to be meaningful enough to be used as regulatory approval endpoints. The FDA approval of the cytotoxic mitoxantrone in 1996 was based upon compelling evidence of palliative benefit which was defined by an improvement in pain, i.e. a two-point reduction in the six-point present pain intensity scale of the McGill–Melzack Pain Questionnaire [57]. This improvement in pain was observed in the absence of a difference in OS. It is of course relevant to consider the state of prostate cancer therapeutics in mid-1990s, with no effective therapy of any kind available following progression on ADT. Although HRQoL measures remain embedded in many phase III trials with some parameters such as pain informing hard endpoints, overall survival has become the defacto endpoint for registration since the approval of docetaxel in 2004 [58].

The use of patient-reported outcome measures as endpoints has again become more relevant with the availability of next generation antiandrogens and immunomodulatory therapy with sipuleucel-T as the potential for use of these therapeutics in a low-symptom burden patient population is increasingly considered. Challenges in the interpretation of these measures include the lack of correlation between patient-reported outcomes and clinically relevant endpoints such as PSA response and pain improvement as well as harder endpoints, i.e. survival.

Incorporating Biomarkers as Endpoints in CRPC

Although PSA is the most widely used biomarker in the management of prostate cancer, its inability to be used as a surrogate for relevant endpoints such as survival has led to a massive effort to identify new biomarkers. While a full discussion of these efforts is well beyond the scope of this chapter, work in the area of circulating tumor cells (CTCs) as potential biomarkers has demonstrated some utility in predicting overall survival

as well as assessing treatment effects, and remains a highly promising area of research. The definition, isolation, and identification in mCRPC has been thoroughly reviewed by others [59, 60]. CTC counts used as a continuous variable is prognostic of survival pre-therapy and changes post-therapy are predictive of both PFS and OS in breast cancer [61]. In prostate cancer, baseline CTC numbers and PSA levels evaluated together are strongly associated with survival. The greater the CTC number the greater the risk of death from mCRPC. In fact CTCs performed better than the traditional PSA decline >50 % parameter with regard to predict overall survival [62, 63]. Early trials with abiraterone and enzalutamide demonstrated the ability to convert unfavorable pretreatment to favorable post-treatment (≥5 cells to <5 cells/7.5 mL) CTC numbers [64, 65].

Prospective CTC assessment has been embedded in most of the recent phase III trials of novel compounds. In the post-docetaxel abiraterone phase III trial CTC levels were assessed at baseline and 4, 8, and 12 weeks' follow-up. CTC conversion using a standard definition for unfavorable (CTC ≥ 5) and favorable (CTC < 5) counts was predictive of OS as early as 4 weeks after treatment [66]. Despite these encouraging findings, we currently remain far short of validating CTC counts as a surrogate endpoint for survival in men with mCRPC.

Conclusions

Substantial progress has been made in the management of mCRPC as a consequence of a rapidly evolving understanding of the biology of the androgen receptor and the rapid translation of these insights into novel therapeutic agents. Over the past few years, five drugs have been approved for the management of mCRPC, all approved by the FDA on the basis of an overall survival endpoint. The recent negative results from the phase III orteronel study, an agent with biologic properties very similar to abiraterone may be the first real indication that while an overall survival endpoint is clean and unambiguous, our recent progress in therapeutics may be beginning to outpace our ability to develop achievable endpoints. The need to develop surrogate endpoints has never been more of an unmet need.

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Part IV

New Drug Development: Specific Targets

Angiogenesis Inhibition in Castration-Resistant Prostate Cancer

15

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Angiogenesis as a Target in Cancer Therapy

Origins of the Anti-angiogenesis Concept

The novel concept of tumor angiogenesis as a therapeutic target in oncology was postulated in 1971 by Judah Folkman. In his seminal thought piece *Tumor Angiogenesis: Therapeutic Implications*, Folkman summarized prior research demonstrating the dependence of tumor growth on the ability of tumor cells to induce neovascularization, providing the rational basis for this process as a therapeutic target. In this work, Folkman also coined the term "anti-angiogenesis" and predicted that "anti-angiogenesis therapy…should provide a powerful adjunct to the control of solid neoplasms" [1]. Over the next several decades, interest in tumor angiogenesis would slowly gain momentum, eventually garnering such expectations from scientists and the public alike that it has been likened to a "Holy Grail" of oncology [2].

It would be many years, however, before the isolation of the individual mediators of angiogenesis would be accomplished, a necessary step for the development of clinical applications. Vascular endothelial growth factor (VEGF) (also known as vascular permeability factor, or VPF), which has become one of the most widely studied angiogenesis stimulating molecules, was not isolated until 1989 [3]. Since then, our understanding of the biochemistry to angiogenesis has expanded greatly, recognizing many other important signaling factors, including transforming growth factors alpha and beta (TGF-α and TGF-β), angiogenin, platelet-derived growth factor (PDGF), tumor necrosis factor alpha (TNF-α), the fibroblast growth factor (FGF) family, and the angiopoietins (ANGs) [4], among others. The current conceptual model, termed the "angiogenic switch," holds that angiogenesis begins when the net balance of pro- and anti-angiogenic factors passes a certain threshold [5].

The relatively small role of angiogenesis in the adult human, in principle, also makes it a tolerable therapeutic target. While very active during the embryogenesis, VEGF levels decline thereafter to near absence from most adult tissues, with preserved expression in select contexts such as gonadal tissues [6] and wound healing [7]. The findings of limited expression of angiogenic factors in most adult tissues suggest that angiogenesis might be inhibited without producing significant toxicity.

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F. Saad and M.A. Eisenberger (eds.), *Management of Castration Resistant Prostate Cancer*, Current Clinical Urology, DOI 10.1007/978-1-4939-1176-9_15,

Development of the Anti-angiogenesis Paradigm

Though the hypothesis of neovascularization as a central driver of tumor growth was physiologically plausible; the high metabolic needs of malignant tumors and the limited diffusion capacity of oxygen through solid tissues require that rapidly growing tumors maintain an active vascular supply. However, additional data were needed to confirm its role in human pathology. Interest in the field of anti-angiogenesis would grow as new research began to link neovascularization directly to worsened outcomes in human cancers.

Some of the first clinical data to support the vital role of angiogenesis in human cancer would come during the mid-1980s from work on melanoma. Srivastava et al. correlated the degree of neovascularization of cutaneous melanoma with the depth of the lesion, suggesting that this process is necessary to reach a threatening level of invasion [8]. Further work by the same group would find a correlation not only between tumor vascularity and depth, but also with risk of recurrence and metastasis [9].

This research soon led to similar investigations in other malignancies, and within a few years tumor neovascularization would be linked to poor outcomes in many other human cancers. Folkman's group correlated microvascular density in the primary lesion with risk of distant metastasis in breast cancer [10]. Similar work during the early 1990s would extend this correlation to malignancies of the lung [11], prostate [12], and bladder [13].

Simultaneously, biochemical research began to decode the mechanisms of tumor neovascularization, finding largely that tumors rely on the same molecular pathways to initiate this process as do healthy tissues. Histopathological studies identified VEGF expression in tumor cells and staining for VEGF protein in adjacent blood vessels, suggesting that VEGF "is synthesized by tumor cells *in vivo* and accumulates in nearby blood vessels, its target of action" [14]. Overexpression of VEGF was found to promote a more aggressive phenotype in human cervical cancer cells [15] as well as additional growth of an experimental ovarian cell line [16]. Further circumstantial evidence was provided by the observation that cancer patients have higher serum levels of VEGF than do healthy controls [17]. The importance of these findings was highlighted by further data from Folkman's group, demonstrating that angiogenesis is a necessary step in the transition from hyperplasia to neoplasia in a model system of pancreatic cancer [18].

From Bench to Bedside

By the early 1990s, development of therapeutic angiogenesis inhibitors was already underway. Proof-of-principle was first demonstrated in vivo when a monoclonal antibody against VEGF-A inhibited growth of a variety of human cancer types in a mouse model [19]. This non-humanized antibody was a precursor to bevacizumab, which would later become the hallmark angiogenesis inhibitor in humans. Further study of bevacizumab's mechanism of action confirmed that it reduces tumor vascularity and blood flow [20], but without direct cytotoxicity to non-diseased cells [21].

Bevacizumab would be submitted as an investigational new drug in 1997. Phase I trials found that bevacizumab added little toxicity to existing chemotherapy regimens, further supporting the notion that angiogenesis might be inhibited with minimal collateral effect on healthy tissues [22]. Bevacizumab would later receive FDA approval in 2004 [23] after phase III trial data showed an improvement in overall survival for metastatic colorectal cancer [24]. Its on-label spectrum has since expanded, receiving approval for treatment of lung, renal, and breast cancer, as well as glioblastoma multiforme [23].

However, bevacizumab has failed to live up to the initial high expectations. While demonstrating improvements in progression-free survival in colorectal, non-small cell lung, breast, and renal cell cancer [24–27], overall survival improved in only trials of colorectal [24] and non-small cell lung cancer [25]. Furthermore, the overall survival benefit for lung cancer was not

re-demonstrated in a later placebo-controlled, rather than open label, trial [28]. Controversially, the FDA later removed the indication for metastatic breast cancer [23].

Regardless of the lukewarm response to bevacizumab, research on the therapeutic inhibition of angiogenic pathways has continued, with multiple new agents under development and newly approved on the market. A variety of tyrosine kinase inhibitors (TKIs), including sunitinib, sorafenib, pazopanib, and cabozantinib, inhibit multiple targets in several different angiogenic pathways, potentially making these agents more potent than narrow-spectrum monoclonal antibodies [29]. The thalidomide family members, which exert an anti-angiogenic effect via unknown mechanisms thought to be the result of immunomodulatory capabilities, are undergoing a resurgence of interest for the treatment of many cancer types [30]. Many additional agents, including several novel small molecules, are still in the early stages of development. Despite nearly a decade of experience with bevacizumab, anti-angiogenic therapies are still in a nascent phase.

Angiogenesis in Prostate Cancer: Preclinical Findings

Histopathologic Evidence for Angiogenesis in Prostate Cancer

Although no anti-angiogenic agents have yet been approved for treatment of prostate cancer [31], the results of multiple pathophysiologic studies present a strong rationale for their use in this malignancy as well.

Areas of tissue hypoxia have been demonstrated within prostate tumors, suggesting that these cancers are, like many others, dependent on neovascularization for growth [32]. The increased microvascularity within prostate tumors is notably different from the surrounding benign tissue, indicating that this change is pathophysiologic [33].

Similar to many other malignancies, an increasing degree of microvascularity within prostate tumors has been associated with a greater risk of metastasis, as well as with a higher Gleason score [12]. Several additional studies have given further support to an association between microvascular density and poor outcomes in prostate cancer. Silberman et al. found that microvascular density and Gleason score were independent predictors of disease recurrence in intermediate-grade prostate tumors undergoing radical prostatectomy [34]. In a later, similar study, Storhmeyer et al. also found that angiogenesis correlated with an increased likelihood of recurrence after radical prostatectomy [35]. Furthermore, a multivariate analysis by Halvorsen et al. found that of many factors tested—including tumor size, capsular penetration, and positive surgical margins—only microvascular density was predictive of clinical recurrence [36]. Microvessel density in prostate cancer has also been shown to predict the hard endpoint of disease-specific survival [37].

Interestingly, more recent analysis has suggested that it may be other characteristics of the prostate tumor microvasculature, rather than the simple number of vessels, that correlate with disease progression. Mucci et al. assessed several different metrics of tumor vessels, with the conclusion that it was not microvessel density, but the smaller size and irregular shape of the microvessels that predicted prostate cancer mortality [38]. The role of disrupted vessel architecture—leading to increased vascular permeability—supports a growing focus on the tumor "microenvironment" [38]. While the demand for metabolites appears to drive neovascularization, it may be the local environment of chemical signals, inflammation, vascular permeability, and loss of cellular adhesion that allow for distant metastasis and hence drive mortality.

Biochemical Pathways in Angiogenesis

Vascular Endothelial Growth Factor

Although there are many signaling pathways that direct angiogenic processes in both healthy and cancerous tissues (Fig. 15.1), the VEGF pathway has become the hallmark. Its downstream targets have been the best characterized, and it has

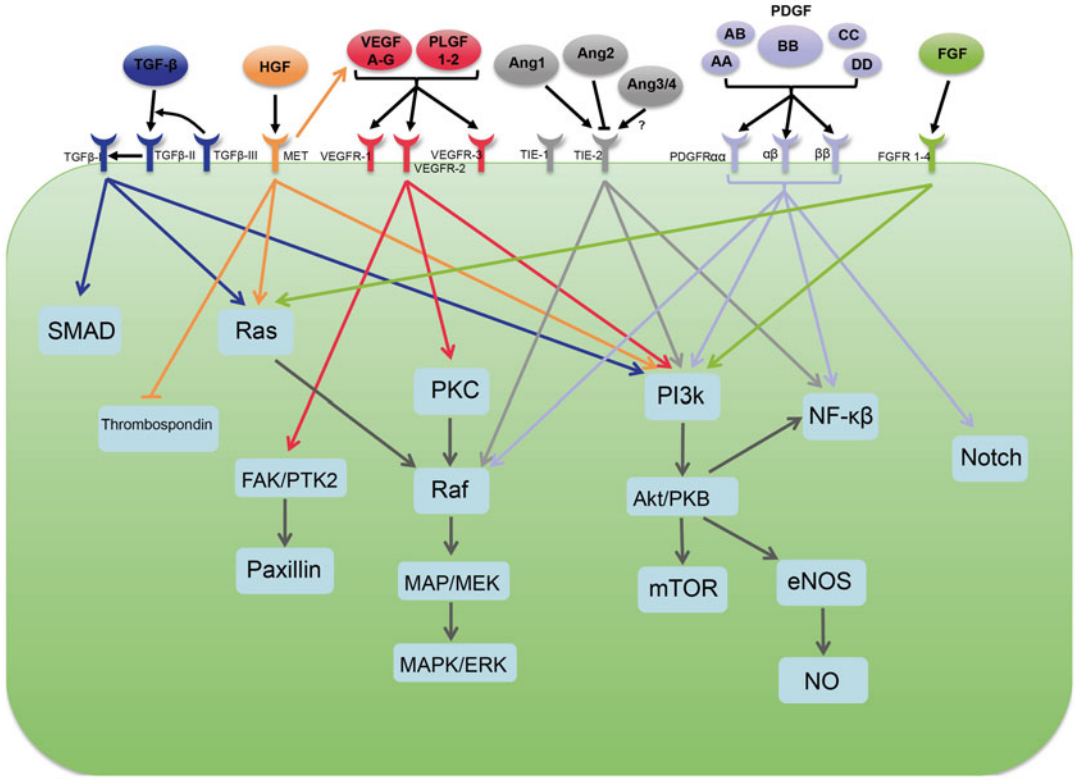

Fig. 15.1 *TGF*-β transforming growth factor-β; *HGF* hepatocyte growth factor; *VEGF* vascular endothelial growth factor; *PLGF* placental growth factor; *Ang* angiopoietin; *PDGF* platelet-derived growth factor; *FGF* fibroblast growth factor

received the most focus in the development of anti-angiogenic therapies.

VEGF is actually a family of seven related growth factors (VEGF-A through -E and placental growth factor [PLGF] 1 and 2), each with varying affinities for the several VEGF receptors. VEGF-A, originally known as vascular permeability factor (VPF), is a central mediator of angiogenesis, and has become a leading therapeutic target. Indeed, in the medical literature "VEGF" is used as synonymous with "VEGF-A."

The secreted VEGF proteins bind to three known receptor tyrosine kinases: VEGFR-1 (Flt-1), VEGFR-2 (KDR/Flk-1), and VEGFR-3 (Flt-4). It is the action of VEGF-2, particularly upon binding the VEGF-A ligand, that has been the best studied in the context of anti-angiogenic therapy [39]. VEGFR-1 and VEGFR-2 expression is restricted largely to vascular endothelial cells, and their actions are relatively specific for this tissue. VEGFR-3, however, is restricted to lymphatic endothelial cells [40], and is felt to play a more important role in lymphangiogenesis than angiogenesis [41], though it has been shown to activate the Ras pathway similarly to VEGFR-2. Furthermore, VEGFR-3 does not bind VEGF-A, but rather VEGF-C and -D. The function of VEGFR-1 is not yet well understood, though it appears to play a role in monocyte migration, and a soluble version of the extracellular VEGFR-1 domain may be a negative regulator of angiogenesis during embryogenesis by acting as a "trap" for VEGF-A ligand [41].

Ligand binding results in homodimerization of VEGFR-2, leading to the activation of multiple intracellular pathways that upregulate expression of genes promoting the growth, survival, and migration of vascular endothelial cells.

Activation of the protein kinase C (PKC)–Raf–MAP/ERK kinase (MEK)–MAPK/ERK pathway promotes endothelial cell growth. VEGFR-2 also activates the phosphatidylinositol 3-kinase (PI3K)–Akt/PKB pathway, which upregulates mTOR, a central driver of cellular proliferation and survival. Additionally, Akt/PKB also results in the phosphorylation of endothelial nitric oxide synthase (eNOS), increasing nitric oxide production and therefore increasing endothelial permeability [42]. Finally, by activating the focal adhesion kinase (FAK, also known as protein tyrosine kinase 2 or PTK2)–paxillin pathway, VEGFR-2 directs the migration and reorganization of endothelial cells to form new vessels [43].

Angiopoietin

Angiopoietin-mediated signaling via the TIE receptor tyrosine kinases is important for both embryonic angiogenesis and adult vascular homeostasis [44]. There is significant functional overlap between angiopoietin and VEGF, in terms of the downstream signals that each activates. Like VEGF-A signaling via VEGFR-2, angiopoietin signaling activates the Raf–MEK–MAPK pathway promoting endothelial cell growth [45]. It also activates the PI3K–Akt pathway, with similar upregulation of mTOR and eNOS, promoting vascular cell survival, proliferation, and permeability [45]. NF-kB is also activated, regulating inflammation [45].

There are two receptors for the angiopoietin family—TIE-1 and TIE-2. TIE-1 is poorly understood; it has no known ligand [46], but clearly plays some important role in embryogenesis, as knockout results in an embryonic lethal phenotype [47]. Additionally, the interaction between the angiopoietin family (Ang 1–4) and TIE-2 is complex. Ang-1 is a TIE-2 agonist. Ang-2, however, has a variable effect on TIE-2 that appears to be environment-dependent, serving as an antagonist in the absence of VEGF-A but as an agonist in the presence of VEGF-A [44]. Ang-3 and Ang-4 have limited homology to Ang-1 and Ang-2; their effects on TIE-2 and downstream angiogenesis pathways seem to be variable and dependent on the context [45].

Platelet-Derived Growth Factor

The platelet-derived growth factor (PDGF) family includes four peptides, A through D, which combine by homo- and hetero-dimerization to form a total of five functional, dimeric ligands: PDGF-AA, PDGF-AB, PDGF-BB, PDGF-CC, and PDGF-DD [48]. PDGF-mediated signaling has been shown to stimulate angiogenesis [49]. The transmembrane, tyrosine kinase receptors that bind the PDGF dimers are constituted from the PDGFR-α and PDGFR-β molecules, and include PDGFR-αα, PDGFR-αβ, and PDGFR-ββ. PDGF-BB is the only form of the protein that is capable of binding all three PDGFR dimers [48], a capability that may have functional significance as PDGF-BB appears to have greater angiogenic potency that the other PDGF dimers [50].

Like both VEGF and angiopoietin, PDGF signaling is also capable of activating both the Raf–MEK–MAPK and the PI3K–Akt pathways controlling cell proliferation and survival [51]. Additionally, PDGF also influences the NF-kB and Notch pathways, which, among other effects, increase cellular production of VEGF as well as matrix metalloproteinase 9 (MMP-9), promoting angiogenesis as well as invasion and metastasis [52]. Though there is a large degree of overlap in the downstream signaling cascades by which VEGF and PDGF act, PDGF-mediated signaling is capable of inducing angiogenesis independent of VEGF [53], highlighting its importance in this process and its potential as a therapeutic target.

Fibroblast Growth Factor

The FGF signaling system includes over 20 growth factors and four known FGF receptors (FGFR 1 through 4). Of this large family, FGF2 is the best-understood growth factor and has become the prototype for studying FGF signaling. FGFR1 is the primary receptor expressed on endothelial cells and mediates the growth, migration, and tubular morphogenesis of these cells, while FGFR2 primarily drives cell motility [54]. The FGF pathways are dependent on heparan sulfate proteoglycans, which act as coreceptors during FGF-FGFR binding [55].

FGF binding to FGFR activates the Ras–MEK–MAPK growth-regulating pathway.

FGF signaling has also been observed to activate PI3K–Akt pathway, though this activity has been demonstrated only in non-vascular cells and its importance to FGF-mediated angiogenesis is unclear [54]. FGF also activates the p38 MAP kinase pathway, although this appears to down-regulate FGF-mediated angiogenesis, serving as a negative feedback mechanism [54]. As is the case for most of the angiogenesis pathways, FGF and FGFR are overexpressed in tumors [55]. There is thought to be a synergistic effect between FGF and VEGF [55] as well as FGF and PDGF [48] in the context of tumor neovascularization. FGF signaling may plan additional roles in prostate cancer specifically, as progressive expression of particular splice variants of FGFR2 has been associated with the development of androgen resistance in human prostate cancer tumor models [56].

Transforming Growth Factor-β

The transforming growth factor (TGF) ligand exists in three isoforms, TGF-β1 through 3. There are three classes of TGF-β receptors, types I–III, each of which has a distinct function in TGF-β signaling. Type III receptors do not directly engage downstream signaling cascades, but are thought to facilitate TGF-β binding to type II receptors; type II receptors then recruit and phosphorylate type I receptors, which in turn activate a group of transcription factors known as SMADs. Believed to be specific to TFG-β signaling, activated SMADs translocate to the nucleus and regulate transcription of genes involved in proliferation, differentiation, and angiogenesis [44].

The role of TGF-β in cancer is complex. SMAD-mediated signaling is generally recognized as apoptosis-inducing, which would have the expected effect of suppressing tumor growth. Indeed, there is some evidence to suggest that TGF-β functions as a tumor suppressor in early oncogenesis [57]. However, TGF-β has many other actions outside of the SMAD pathway, including activation of the Ras-Raf-MEK-MAPK and PI3K-Akt cascades that seem to serve as the "final common pathway" for many of the angiogenesis-inducing growth factors [58, 59]. Interestingly, tumorigenesis is often marked by both increased expression of TGF-β and loss-of-function mutations in the SMAD proteins [57], a sequence of events that may result in the preservation of the oncogenic effects of TGF-β within the tumor while avoiding its anti-proliferative functions.

Hepatocyte Growth Factor

Hepatocyte growth factor (HGF), also known as scatter factor (SF), controls a diversity of cellular processes. Its receptor is the transmembrane tyrosine kinase MET (c-MET, HGF receptor). Ligand binding induces MET homodimerization and autophosphorylation, activating the intracellular domain. HGF–MET signaled is primarily mediated by downstream activation of the Ras–Raf–MEK–MAPK and the PI3K–Akt pathways [60].

HGF has long been known to stimulate angiogenesis in vitro [61] and in vivo [62]. HGF–MET signaling may produce this effect via indirect action on other pathways. Specifically, HGF signaling leads to the inhibition of thrombospondin 1, a negative regulator of angiogenesis; HGF also induces VEGFA production, thereby upregulating one of the most potent angiogenic pathways [63].

Additionally, HGF–MET signaling is one of the pathways most clearly associated with the process of epithelial–mesenchymal transition (EMT). Normal EMT during embryogenesis allows for the migration of precursor cells, and is dependent on HGF–MET [60]. Similarly, EMT in the context of malignancy has been proposed as a central process in the development of metastatic capability [64]. The role of HGF–MET signaling has been well documented in many malignancies, including hepatocellular carcinoma [65], where the inhibition of HGF–MET–mediated EMT has been proposed as a possible mechanism of action of sorafenib in the treatment of this malignancy [66]. EMT in prostate cancer progression is understood to be induced by local factors in the tumor microenvironment, where it has been demonstrated to promote cell migration, invasion, and survival [64].

Specific Angiogenic Factors in Prostate Cancer

The biochemical mapping of angiogenesis pathways has also led to an increasing understanding

of which signaling factors and pathways are involved in prostate cancer specifically. As in other cancers, VEGF appears to be one of the central players in prostate cancer angiogenesis. Beginning in the year 1997, immunohistochemical analysis of prostate tumors found high levels of VEGF expression in diseased tissue, compared with little or no expression in the normal prostate [67, 68]. Additional in vivo studies would confirm this result, and also demonstrate the absence of VEGF expression in benign prostatic hypertrophy [69]. Coexpression of the VEGF and VEGF receptor in prostate cancer microvasculature has also been reported [70].

VEGF expression in prostate cancer has since been correlated not only with angiogenesis, but also with outcomes. VEGF expression was found to correlate with risk of progression in one cohort [71]. Microvascular density in prostate cancer has also been associated with higher Gleason score and pathologic stage [72]. Findings such as these have led to the proposed use of angiogenesis as an additional prognostic variable in the examination of biopsy specimens. Furthermore, VEGF may have additional use as a circulating biomarker. High urine levels of VEGF may be an independent predictor of survival in the setting of castrate-resistant prostate cancer (CRPC); [73] though this correlation has not held up in other studies [74]. Elevated plasma VEGF levels have also correlated with poor outcomes [75]. These findings support a pathophysiologic role for VEGF in prostate cancer, and suggest that it may be a valid chemotherapeutic target.

Other angiogenic pathways are also important in prostate cancer. Introduction of neutralizing antibodies to the proteins secreted by cultured prostate cancer cells has found that FGF-2, in addition to VEGF, is partially responsible for the angiogenesis-inducing capability of these cells [76]. Immunohistochemical analysis of prostate tumors also demonstrates the activity of the FGF family, showing the coexpression of FGF-2 and the FGF receptor in diseased, but not normal, prostate tissue [77]. TGF-β overexpression has been linked not only with increased angiogenesis, but also with metastasis and poor patient outcomes [78]. Angiopoietin-2 also appears to play a role, as inhibition of its activity in a mouse prostate tumor model reduces microvascular density, cell proliferation, and serum PSA, with prolongation in survival [79]. Inhibition of angiopoietin-2 also increases expression of hypoxia-inducible factor-1α (HIF-1α) RNA, suggesting that the reduction in microvascular density results in ischemia within tumor tissue.

Intriguingly, the effects of androgen signaling, a central driver of prostate cancer growth, may be due in part to its stimulation of angiogenesis. In vitro assays have shown an increase in expression of both VEGF and the VEGF receptor in response to androgens [80]. This functional overlap suggests the potential for a synergistic effect from co-suppression of androgen and angiogenesis signaling pathways.

Potential Benefits of Anti-angiogenic Therapy in Combination Regimens

In prostate as well as other cancers, anti-angiogenic therapy has the potential to augment the effectiveness of other, traditional chemotherapeutics. While this effect is not completely understood, several potential mechanisms have been proposed. Tumor tissue has been described as having elevated interstitial fluid pressure, preventing adequate diffusion of pharmacologic agents; anti-angiogenic agents—specifically bevacizumab [81]—can act to reduce the intra-tumor hydrostatic pressure, thereby increasing drug delivery [82]. This effect may be mediated by promoting the normalization of the tumor microvasculature [83], again emphasizing the importance on the tumor microenvironment in determining its behavior. Normalization of the microvasculature may also serve to increase delivery of cytotoxic agents by decreasing vascular permeability and reducing the non-therapeutic extravasation of drug into the interstitial space [84]. Anti-angiogenic agents may also slow tumor growth by inhibiting autocrine and paracrine signaling [85]. Additionally, traditional chemotherapeutics and anti-angiogenic agents may produce combined effects simply by acting independently on different signaling pathways [85].

With regard to prostate cancer specifically, it is known that androgen signaling promotes tumor

growth in part by stimulating angiogenesis. Introduction of androgens increases VEGF and VEGF receptor expression in prostate model cells [80]. Conversely, androgen blockade has been demonstrated to reduce VEGF expression in in vivo prostate cancer models, with concomitant reductions in microvascular density and tumor burden [86]. Androgen deprivation also prevents the hypoxia-induced neovascularization response within prostate tumors [87]. Findings such as these raise the possibility of a synergistic effect from combined blockade of androgen and angiogenic pathways.

Androgen signaling produces angiogenesis, at least in part, via the hypoxia-induced factor (HIF) pathway. Indeed, one of the processes leading to castrate resistance is the intra-tumoral upregulation of HIF-1, allowing the tumor to respond to reduced levels of androgen and thus survive in an androgen-deprived environment. As many anti-angiogenic agents inhibit the HIF-1 pathway, they may block this resistance mechanism and thus potentiate the effect of androgen blockade [88].

Anti-angiogenic Therapy in Prostate Cancer: Clinical Applications

Targeting the VEGF Pathway in Prostate Cancer

Bevacizumab

Preclinical data on bevacizumab for prostate cancer showed successful suppression of angiogenesis and proliferation of prostate cancer cell lines in vitro [89]. However, early clinical trial data failed to show significant activity. In the first phase II results for bevacizumab in prostate cancer, from an open-label trial of 15 patients with CRPC receiving bevacizumab monotherapy reported in 2001, no patient achieved a complete or partial response (Table 15.1). The best response was a "possible mixed response," occurring in three patients; no PSA responses >50 % were observed [90].

It took several years for interest in bevacizumab for prostate cancer to return, but eventually the research focus shifted to combination chemotherapy, with more success. In 2008, a phase II trial of combination bevacizumab plus docetaxel in 20 patients previously treated with docetaxel reported a >50 % PSA decline in 55 % of patients [91]. Thereafter, a larger phase II trial of bevacizumab in combination with docetaxel and estramustine for CRPC found more encouraging results, with 75 % of its 79 patients achieving a >50 % PSA response, and 59 % of those with measurable disease having a partial response [92]. More recently, bevacizumab has been combined with docetaxel in the neoadjuvant setting before prostatectomy for high-risk localized disease, achieving pre-surgery reductions in tumor volume and PSA, but not yet with long-term follow-up on surgical cure rates [93]. Bevacizumab has also been tested in combination with sipuleucel-T, though more data are needed to test the hypothesis that bevacizumab may potentiate the response to this and other immunotherapies [94].

Based on these phase II results, a placebo-controlled phase III trial randomizing CRPC patients to docetaxel and prednisone with or without bevacizumab was undertaken (CALGB 90401). 1,050 patients were enrolled. There was no difference in the primary endpoint of overall survival (OS) (22.6 months in the bevacizumab arm vs. 21.6 months in the placebo arm, $p=0.181$), although secondary end points of median progression-free survival (PFS) (9.9 vs. 7.5 months, $p<0.0001$), PSA response, and objective response favored the bevacizumab group. Additionally, bevacizumab appeared to increase toxicity, with an increase in treatment-related deaths (3.8 vs. 1.1 %) [95].

There were several reasons why the CALGB 90401 trial may have produced divergent results for PFS and OS, including a greater burden of comorbidities in the bevacizumab arm [96], as well as not continuing bevacizumab treatment beyond the completion of docetaxel therapy, resulting in shorter treatment durations [97]. In subgroup analysis, those patients with poor prognostic factors (such as elevated LDH and alkaline phosphatase) derived greater benefit from bevacizumab treatment. Additionally, anti-VEGF therapies such as bevacizumab may provide a far greater benefit to those patients whose tumors, and trials that are not enriched for such patients may be underpowered to detect this benefit.

Table 15.1 Phase II and III trials of bevacizumab for prostate cancer with reported results

Study title	Phase	N	Primary end point and results	Year	Reference
A phase II trial of humanized anti-vascular endothelial growth factor antibody for the treatment of androgen-independent prostate cancer	II	15	Objective tumor response, PSA response 0 % objective tumor response, 27 % had PSA decline of >50 %	2001	Reese et al. The Prostate Journal, 3: 65–70
Combination of bevacizumab and docetaxel in docetaxel-pretreated hormone-refractory prostate cancer	II	20	Objective tumor response, PSA response 37.5 % objective partial response, 55 % had PSA decline of >50 %	2008	Di Lorenzo et al. [91]
A phase II study of estramustine, docetaxel, and bevacizumab in men with castrate-resistant prostate cancer: results of cancer and leukemia group B (CALGB) 9006	II	79	Progression-free survival Median progression-free survival was 8 months. Median overall survival was 24 months	2011	Picus et al. [92] NCT00016107
Phase 2 study of neoadjuvant docetaxel plus bevacizumab in patients with high-risk localized prostate cancer: a prostate cancer clinical trials consortium trial	II	41	Partial response by endorectal MRI 29 % experienced >50 % reduction in tumor volume	2012	Ross et al. [93] NCT00321646
Combination immunotherapy with prostatic acid phosphatase pulsed antigen-presenting cells (provenge) plus bevacizumab in patients with serologic progression of prostate cancer after definitive local therapy	II	22	PSA response 5 % had PSA decline of >50 %	2006	Rini et al. [94]
Randomized, double-blind, placebo-controlled phase III trial comparing docetaxel and prednisone with or without bevacizumab in men with metastatic castration-resistant prostate cancer: CALGB 90401	III	1,050	Overall survival Overall survival was 22.6 months in the docetaxel + prednisone + bevacizumab group, compared to 21.5 months for the docetaxel + prednisone group ($p = 0.181$)	2012	Kelly et al. [95] NCT00110214
Phase II trial of bevacizumab, thalidomide, docetaxel, and prednisone in patients with metastatic castration-resistant prostate cancer	II	60	PSA response 90 % had PSA decline of >50 %	2010	Ning et al. [98] NCT00091364

Analysis of other trial cohorts (CALGB 9480) has identified elevated plasma VEGF levels as an independent poor prognostic factor [75], suggesting the possibility of selecting for patients with VEGF-dependent biology in future trials.

Since then, combination therapy with bevacizumab for CRPC has taken new directions. A phase II trial of bevacizumab in combination with thalidomide, prednisone, and docetaxel achieved a 90 % rate of >50 % PSA reduction, though this combination was limited by toxicities such as bone marrow suppression [98]. A similar regimen of bevacizumab, lenalidomide, prednisone, and docetaxel is currently under investigation [NCT00942578]. Other ongoing clinical trials are expected to report for the first time on bevacizumab in combination with the mTOR inhibitors everolimus [NCT00574769] and temsirolimus [NCT01083368]. Also of notable interest due to the theoretical synergistic effect [88], several trials are underway to test combined androgen and angiogenesis suppression. One phase II trial aims to test androgen blockade with bicalutamide with or without bevacizumab as first-line therapy after PSA recurrence following prostatectomy [NCT00776594]. Another ongoing phase II trial will evaluate bevacizumab and androgen deprivation with docetaxel in a similar first-recurrence setting [NCT00658697]. However, without study designs enriching for

patients with VEGF-dependent tumor biology, these trials are less likely to achieve new results that will significantly advance the field.

Aflibercept

Aflibercept also blocks the VEGF pathway, but by a different mechanism than bevacizumab; containing regions of the VEGF receptors 1 and 2, this protein acts as a soluble VEGF "trap." Phase I studies were promising, showing signals of activity when used in combination with docetaxel for a variety of cancer types, including prostate cancer [99]. Based on these data, aflibercept was taken directly to the phase III stage, being tested with docetaxel and prednisone in the >1,200 patient VENICE trial [NCT00519285] (Table 15.1). In the VENICE trial, aflibercept did not improve the primary outcome of overall survival compared to placebo, and was associated with an increased risk of multiple toxicities [100]. Similarly to CALGB 90401, the VENICE trial did not enrich its study cohort for poor prognostic factors or elevated VEGF levels, leaving open the possibility that a clinically significant benefit may be possible in a selected cohort with these features. While trials continue to search for new applications for bevacizumab in the treatment of CRPC, the role of aflibercept in the future treatment of this disease remains unclear.

Immunomodulatory Agents: The Thalidomide Family

Thalidomide

Although its potential harms as a devastating teratogen were already known, thalidomide was also demonstrated to be a potent anti-angiogenic factor in the mid-1990s. By use of a rabbit cornea micropocket assay, thalidomide was shown to inhibit FGF-mediated angiogenesis [101]; subsequent research would also show its ability to prevent tumor growth in a rabbit model [102]. The mechanism of the thalidomides' anti-angiogenic activity is not fully understood. Reduction in angiogenic factors such as VEGF and FGF has been observed, along with a pro-apoptotic effect [103]. Additionally, thalidomide has a significant immunomodulatory effect via inhibition of TNF-α, which is also felt to contribute to its anti-angiogenic capability [44].

Since the discovery of its anti-angiogenic properties, thalidomide and its derivatives have become the foundation of treatment for multiple myeloma, significantly improving outcomes in that disease. Applications for prostate cancer have begun to be explored, as well.

Phase II data for prostate cancer began to be reported in 2001 (Table 15.2). A 63-patient trial of thalidomide in combination with docetaxel for CRPC observed a >50 % PSA reduction in 18 % of patients, but the response rate did not appear to increase with higher doses of thalidomide [104]. Several years later, however, a randomized, open label trial of docetaxel with or without thalidomide produced the encouraging result of a significant improvement in overall survival (25.9 months for thalidomide vs. 14.7 months for placebo, $p = 0.0407$) [105]. Venous thromboembolism was a significant adverse reaction to this regimen, which was effectively prevented later in the trial by the institution of low-molecular weight heparin for all participants. Thalidomide has also undergone phase II testing in combination with docetaxel and estramustine, which achieved a >50 % PSA response in 90 % of subjects and a PFS of 7.2 months [106].

The only double-blinded, randomized data on thalidomide for prostate cancer comes from a phase III trial of recurrent, but not castrate-resistant, disease. This study randomized patients with recurrent prostate cancer to androgen deprivation therapy followed by thalidomide or placebo, with a primary end point of time to PSA progression; this process was repeated at first evidence of progression, to allow for two treatment-and-progression phases. Though a trend towards a longer progression-free interval was present for both treatment phases, the difference was statistically significant for only the second phase (17.1 months for thalidomide vs. 6.6 months for placebo, $p = 0.0002$) [107].

Thalidomide has also been studied as part of a potent combination with bevacizumab, prednisone, and docetaxel. This regimen achieved a 90 % rate of >50 % PSA reduction, though it was not suitable for further study due to bone marrow suppression [98]. Due to a better side

Table 15.2 Phase II and III trials of thalidomide and lenalidomide for prostate cancer with reported results

Study title	Phase	N	Primary end point and results	Year	Reference
Thalidomide					
A randomized phase II trial of thalidomide, an angiogenesis inhibitor, in patients with androgen-independent prostate cancer	II	63	Primary end point not specified 0 % had an objective partial response, 14 % had PSA decline of >50 %	2001	Figg et al. [104]
Randomized phase II trial of docetaxel plus thalidomide in androgen-independent prostate cancer	II	75	Overall survival and progression-free survival Median progression-free survival was 3.7 months in the docetaxel group and 5.9 months in the docetaxel + thalidomide group ($p = 0.32$). 18-month overall survival was 42.9 % in the docetaxel group and 68.2 % in the docetaxel + thalidomide group ($p = 0.11$)	2004	Dahut et al. [105] NCT00020046
Preclinical and clinical evaluation of estramustine, docetaxel and thalidomide combination in androgen-independent prostate cancer	II	20	Progression-free survival, objective response, PSA response Progression-free survival was 7.2 months. 20 % had a partial radiographic response. 90 % had a PSA decline of >50 %	2007	Figg et al. [106] NCT00083005
A double-blind randomized crossover study of oral thalidomide versus placebo for androgen-dependent prostate cancer treated with intermittent androgen ablation	III	159	Biochemical progression-free survival Median time to PSA progression was 15 months in the thalidomide group compared to 9.6 months in the placebo group ($p = 0.21$)	2009	Figg et al. [107] NCT00004635
Phase II trial of bevacizumab, thalidomide, docetaxel, and prednisone in patients with metastatic castration-resistant prostate cancer	II	60	PSA response 90 % had PSA decline of >50 %	2010	Ning et al. [98] NCT00091364
Lenalidomide					
Sargramostim (GM-CSF) and lenalidomide in castration-resistant prostate cancer (CRPC): Results from a phase I–II clinical trial	I–II	32	Objective tumor response, PSA response, and safety 18 % objective response rate. 13 % with PSA decline of >50 %. 22 % experience grade 3–4 toxicity	2013	Garcia et al. [111] NCT00939510
A phase 3 study to evaluate the efficacy and safety of Docetaxel and Prednisone (DP) with or without Lenalidomide (LEN) in patients with castrate-resistant prostate cancer (CRPC): the MAINSAIL trial	III	1,059	Overall survival Median overall survival was 77 weeks in the docetaxel + prednisone + lenalidomide group, compared to median not reached in the docetaxel + prednisone + placebo arm ($p = 0.0017$)	2011	Petrylak et al. [112] NCT00988208

effect profile, interest and research efforts have shifted towards the thalidomide derivative lenalidomide [108].

Lenalidomide

Lenalidomide has a more tolerable side effect profile than thalidomide. This became clear during development for myeloma treatment, and has been confirmed in phase I dose-escalation trials of prostate cancer patients [109]. Since then, research into this new member of the thalidomide family has grown, with several trials currently underway (Table 15.2).

An earlier phase II trial suggested that a combination regimen of thalidomide, bevacizumab, prednisone, and docetaxel was highly active but

limited by toxicity [98]. To build on this result, and hopefully to reduce toxicity with the substitution of thalidomide for lenalidomide, a phase II trial of lenalidomide, bevacizumab, prednisone, and docetaxel has been undertaken [NCT00942578]. This trial is still ongoing, but preliminary results have reported response rates of 79.3 % by radiography and 86.7 % by PSA [110]. Another phase I–II trial combining GM-CSF with lenalidomide demonstrated a favorable toxicity profile but only modest anti-tumor activity, with 12.5 % of CRPC patients achieving a PSA response of >50 % and 18 % of those with measurable disease having a radiographic response [111].

To try to replicate the potential favorable results observed with thalidomide plus docetaxel, a phase III trial was undertaken to test lenalidomide in combination with docetaxel for the treatment of CRPC. This multicenter, randomized, double-blinded trial, known as the MAINSAIL trial, enrolled a total of 1,059 patients. Disappointingly, the MAINSAIL trial was terminated early due to lack of efficacy, as lenalidomide failed to improve the primary outcome of overall survival [112].

Despite this significant setback, evaluation of other potential uses for lenalidomide in CRPC is ongoing. Lenalidomide is currently being studied in combination with paclitaxel [NCT00933426] as well as cyclophosphamide [NCT01093183] for the treatment of this disease.

Anti-angiogenic Tyrosine Kinase Inhibitors

Sunitinib

Many of the small-molecule TKIs act upon angiogenic pathways. They have the theoretical potential for a greater anti-angiogenic activity than single-target agents such as bevacizumab, as they often block multiple signaling pathways that contribute to angiogenesis. This fact also makes a broader range of side effects more likely, however. TKIs target tumor cells as well as the endothelial cells, turning off the autocrine and paracrine signals that promote tumor neovascularization [31].

Sunitinb malate inhibits a number of pro-angiogenic targets, including the VEGF receptors, PDGF receptors, the tyrosine-protein kinase KIT, colony stimulating factor 1 receptor, and receptor tyrosine kinases encoded by c-RET [31]. With FDA approval for the treatment of renal cell cancinoma and pancreatic neuroendocrine tumors, sunitinib is also one of the best-studied TKIs for the treatment of CRPC.

Anti-prostate cancer activity of sunitinib has been observed in several phase II trials (Table 15.3). The first phase II results for CRPC, reported in 2009, showed few PSA responses (2 of 34 patients, the primary end point), though it was noticed that radiographic responses were often discordant with PSA responses, raising the possibility that sunitinib's anti-tumor activity was not being adequately captured by the PSA endpoint [113]. A similar trial of sunitinib monotherapy for CRPC after failure of cytotoxic chemotherapy also showed a low rate of >50 % PSA responses (12.1 %), though large numbers of patients saw smaller declines by both PSA and radiographic criteria [114]. Additional encouraging results were seen in combination with docetaxel and prednisone for chemotherapy-naïve CRPC, with a PSA response rate of 56.4 % and 12.6-month average PFS [115].

Based on these results, a placebo-controlled phase III trial of sunitinib in combination with prednisone for CRPC previously treated with docetaxel was undertaken [NCT00676650]. Unfortunately, this trial was stopped prematurely based on preliminary results indicating that the primary endpoint of overall survival would not show a benefit. Similar to the CALBG 90401 trial of bevacizumab for CRPC [95], sunitinib showed a significant improvement in PFS (5.6 vs. 3.7 months, $p=0.0077$) but no improvement in overall survival (13.1 vs. 12.8 months, $p=0.58$) [116].

Despite this setback, sunitinib is still undergoing active study for the management of prostate cancer. A single-arm phase II trial is examining sunitinib in the non-CRPC setting, as part of combined treatment with docetaxel and salvage radiation therapy for PSA recurrence after prostatectomy [NCT00734851. Sunitinib is also being tried in the neoadjuvant setting before

Table 15.3 Phase II and III, and selected phase I trials of TKIs for prostate cancer with reported results

Study title	Phase	N	Primary end point and results	Year	Reference
Sunitinib					
Phase II study of sunitinib in men with advanced prostate cancer	II	34	PSA response 6 % had PSA response >50 % regardless of docetaxel-naïve status	2009	Dror Michaelson et al. [113] NCT00299741
Sunitinib malate for metastatic castration-resistant prostate cancer following docetaxel-based chemotherapy	II	36	Progression-free survival Median progression-free survival was 19.4 weeks	2010	Sonpavde et al. [114]
Sunitinib in combination with docetaxel and prednisone in chemotherapy-naive patients with metastatic, castration-resistant prostate cancer: a phase 1/2 clinical trial	I–II	55	PSA response 56 % had PSA decline of >50 %	2012	Zurita et al. [115] NCT00137436
A multicenter, randomized, double-blind, phase 3 study of sunitinib plus prednisone versus prednisone in patients with progressive metastatic castration-resistant prostate cancer after failure of a docetaxel-based chemotherapy regimen	III	873	Overall survival Trial was stopped early for futility. At the time of discontinuation, disease progression rates were 28.1 % in the sunitinib group and 47.3 % in the placebo group	2010	Ou et al. [116] NCT00676650
Sofafenib					
A clinical phase II study with sorafenib in patients with progressive hormone-refractory prostate cancer: a study of the CESAR Central European Society for Anticancer Drug Research-EWIV	II	47	12-week progression-free survival Median progression-free survival was 8 weeks	2007	Steinbild et al. [118]
A phase II study of BAY 43-9006 (Sorafenib) in metastatic, androgen-independent prostate cancer	II	22	Progression (radiographic, biochemical, or symptomatic) Median progression-free survival was 1.8 months. No responses were seen by radiographic or biochemical measures	2008	Dahut et al. [119] NCT00090545
Final analysis of a phase II trial using sorafenib for metastatic castration-resistant prostate cancer	II	24	Progression-free survival Median progression-free survival was 3.7 months and median overall survival was 18.0 months	2009	Aragon-Ching et al. [120] NCT00090545
A phase II study of sorafenib in patients with chemo-naive castration-resistant prostate cancer	II	28	PSA response 3.6 % had PSA decline of >50 %	2008	Chi et al. [121]
A phase II study of sorafenib in combination with bicalutamide in patients with chemotherapy-naive castration-resistant prostate cancer	II	39	PSA response 32 % had PSA decline of >50 %	2012	Beardsley et al. [122]

(continued)

Table 15.3 (continued)

Study title	Phase	N	Primary end point and results	Year	Reference
Cabozantinib					
Cabozantinib in patients with advanced prostate cancer: results of a phase II randomized discontinuation trial	II	171	Objective response rate and progression-free survival Median progression-free survival was 23.9 weeks with cabozantinib compared to 5.9 weeks in the placebo group ($p<0.001$)	2013	Smith et al. [123] NCT00940225
Vandetanib					
A phase II, double-blind, placebo-controlled, randomized study to assess the efficacy and safety of docetaxel (taxotere)/prednisolone/ZD6474 vs docetaxel/prednisolone/ placebo in patients with hormone-refractory prostrate cancer (HRPC)	II	86	PSA response 40 % of subjects had a >50 % decline in PSA in the docetaxel + prednisolone + vandetanib group, compared to 67 % in the docetaxel + prednisolone group	2011	NCT00498797
Efficacy and Safety of Zactima™ in patients with castration-refractory metastatic prostate cancer	II	110	PSA progression-free survival at 4 months PSA progression-free survival was 18 % in the bicalutamide + vadetanib group and 16 % in the bicalutamide + placebo group	2011	NCT00659438
Pazopanib					
Pazopanib hydrochloride after leuprolide acetate or goserelin acetate in treating patients with relapsed prostate cancer	II	37	Time to PSA progression Trial was stopped early due to high drop-out rates	2012	Ward et al. [128] NCT00454571
Cediranib					
Phase I dose-escalation and pharmacokinetic study of AZD2171, an inhibitor of the vascular endothelial growth factor receptor tyrosine kinase, in patients with hormone-refractory prostate cancer (HRPC)	I	26	Maximum tolerated dose Maximum tolerated dose was found to be 20 mg per day. PSA reductions were seen in 15 % of patients	2007	Ryan et al. [130]

prostatectomy [NCT00329043]. A randomized phase II is comparing sunitinib vs. dasatinib, plus abiraterone and prednisone, for control of CRPC [NCT01254864].

Sorafenib

Sorafenib is another small-molecule, multityrosine kinase inhibitor with a broad spectrum of activity. Anti-angiogenic activity is thought to derive from its inhibition of the VEGF receptors 2 and 3 and PDGF receptor β, as well as p38, c-kit, b-Raf, and c-Raf. By these pathways, sorafenib also exhibits direct anti-tumor and pro-apoptotic as well as anti-angiogenic effects [117].

Beginning in 2007, several phase II trials have evaluated sorafenib for CRPC (Table 15.3). When used as monotherapy for CRPC, the best response by RECIST was stable disease in 4 of 55 patients; two patients were responders by PSA, and 31 % overall met the primary endpoint of PFS at 12 weeks [118]. Another trial of sorafenib monotherapy for CRPC noticed a discordance between radiographic and PSA response; while most patients (21 of 22) progressed, 13 of 21 progressed only by PSA in the absence of any radiographic progression. Additionally, two patients initially experienced a remarkable reduction in bony metastatic disease [119]. After these encouraging results, patient accrual was continued; final results later reported 1 partial response and 11 with stable disease out of 24 total patients [120]. A separate trial of sorafenib monotherapy for CRPC also noticed a poor PSA response rate, with only 1 of 28 patients having a >50 % decline; however, in this trial most of the patients showed radiographic progression as well [121]. Sunitinib has been tested in combination with androgen deprivation therapy (bicalutamide) for CRPC with better results—nearly half (18 of 39) of patients achieved the primary outcome of either a PSA response or stable disease at >6 months [122]. As many of these patients had previously progressed on anti-androgen therapy, including bicalutamide, these results raise the possibility of synergistic effect of anti-androgen and anti-angiogenic therapy. However, this possibility has not yet undergone further testing, and there are not currently any ongoing trials of sorafenib in prostate cancer.

Cabozantinib

Cabozantinib is a small-molecule TKI that exerts anti-angiogenic action via inhibition of the VEGF receptor 2 and MET (the HGF receptor), a promoter of tumor invasion and metastasis [31]. It has additional anti-tumor effects via inhibition of c-ret and c-kit. Results from several ongoing trials of this agent are highly anticipated, after a remarkable response rate seen in early phase II data (Table 15.3). Cabozantinib has already been FDA-approved for the treatment of medullary thyroid cancer.

To date, only one phase II trial of cabozantinib for CRPC has been reported. In a randomized discontinuation design, 171 patients with CRPC were treated with cabozantinib monotherapy for a 12-week lead-in phase, during which 72 % of the 154 patients with measurable disease saw regression of soft tissue lesions; those 31 patients who had stable disease after the lead-in phase were randomly assigned to continue cabozantinib or to placebo. The trial was stopped early due to benefit; PFS during the post-randomization period was 23.9 weeks for cabozantinib vs. 5.9 weeks for placebo ($p<0.001$). Additionally, significant reductions were seen in the secondary outcomes of bone pain and narcotic use [123].

After preliminary data from this trial were reported at the American Society of Clinical Oncology meeting in 2011, multiple trials have begun to expand upon these exciting results. The COMET-I trial, a phase III, double-blinded, placebo-controlled trial comparing cabozantinib to prednisone in the setting of previously treated CRPC is ongoing [NCT01605227]; COMET-II, comparing cabozantinib to combined mitoxantrone and prednisone for previously treated CRPC with the primary endpoint of pain response is currently recruiting patients [NCT01522443]. Additional phase II trials are ongoing as well, evaluating cabozantinib monotherapy in CRPC with visceral [NCT01834651] and bony [NCT01428219] metastasis, in non-metastatic disease [NCT01703065], in combination with abiraterone [NCT01995058], and also as first-line treatment in combination with anti-androgen therapy for metastatic but castrate-naïve prostate cancer [NCT01630590].

Vandetanib

Vandetanib is a small-molecule TKI that has been employed in the treatment of medullary thyroid carcinoma, where its activity is thought to derive from its inhibition of the RET oncogene. It also has anti-angiogenic activity via inhibition of the VEGF receptors 2 and 3, as well as the epidermal growth factor (EGF) receptor [124]. Vandetanib has also been studied in other malignancies, with a phase III trial for advanced non-small cell lung cancer showing a modest benefit in PFS (4.0 vs. 3.2 months for placebo, $p < 0.0001$) but no benefit OS [125].

The first data for vandetanib in prostate cancer came in 2007; a randomized, double-blinded phase II trial tested vandetanib in combination with docetaxel and prednisolone for chemotherapy-naïve CRPC (Table 15.3). Forty three patients were enrolled into each group; more patients in the vandetanib group withdrew (38) compared to placebo (29), which was driven by a higher rate of adverse events. The primary end point of PSA response was more common in the placebo group (29 patients vs. 17 patients for vandetanib) [NCT00498797]. Vandetanib was also tried in combination with bicalutamide for CRPC, without improvement in the primary endpoint of PSA progression, or in PFS or OS [NCT00659438]. There are no ongoing clinical trials of vandetanib for prostate cancer.

Pazopanib

This TKI is active on several angiogenic targets, including VEGF receptors 1–3 signaling, PDGF receptors α and β, and the FGF receptor [126]. It has been approved for use in advanced soft tissue sarcoma and in advanced renal cell carcinoma after phase III results showed significant improvement in PFS [127].

Several early trials of pazopanib in prostate cancer met significant hurdles (Table 15.3). One phase II trial of pazopanib for castrate-sensitive prostate cancer was terminated due to high drop-out rates, driven by toxicity in the pazopanib arm and protocol non-compliance in the placebo arm [128]. Another phase II trial of pazopanib in CRPC was terminated due to slow enrollment [NCT00945477]. Despite these setbacks, research in this area is ongoing. A randomized, double-blinded phase I/II combining pazopanib with docetaxel and prednisone in the setting of CRPC with unfavorable risk factors is currently recruiting [NCT01385228]. A phase II of neoadjuvent pazopanib before prostatectomy with a primary endpoint of risk of metastasis is planned but has not yet opened for recruitment [NCT01832259].

Cediranib

Cediranib has a large degree of overlap in its inhibitory spectrum with pazopanib, targeting the VEGF receptors 1–3, PDGF receptors α and β, and the FGF receptor, as well as c-KIT [129].

Early dose-escalation studies in prostate cancer reported 5 PSA reductions out of 26 patients, noting that these responses were often delayed, occurring after drug withdrawal (Table 15.3) [130]. Phase II data is still being awaited. A randomized phase II of docetaxel and prednisone with or without cediranib for CRPC is ongoing [NCT00527124]. Cediranib is also undergoing evaluation as monotherapy in CRPC previously treated with docetaxel [NCT00436956].

Other Classes and Novel Agents

Tasquinimod

Tasquinimod is a quinoline-3-carboxamide derivative which has been found to have anti-angiogenic effects in prostate cancer model systems. Its mechanism of action, elucidated using an in vitro prostate cancer model appears to be upregulation of thrombospondin-1, an inhibitor of HIF-1, resulting in a down-regulation of VEGF production [131]. This novel mechanism of action makes this a particularly interesting agent, especially as it may allow for combination therapy with other drugs targeting the VEGF pathway at multiple points.

A single phase II clinical trial has been reported, and its results were encouraging (Table 15.4). This relatively large (201 patients), randomized, double-blinded study of tasquinimod for chemotherapy-naïve, metastatic CRPC found a significant improvement in PFS (7.6 vs. 3.3 months for placebo, $p = 0.0042$) [132].

Table 15.4 Phase II and III trials of novel angiogenesis inhibitors for prostate cancer

Study title	Phase	N	Primary end point and results	Year	Reference
Everolimus					
RAD001 in patients with metastatic, hormone-refractory prostate cancer	II	35	PSA response 0 % had PSA decline >50 %	2013	NCT00629525
Phase 2 trial of single-agent everolimus in chemotherapy-naive patients with castration-resistant prostate cancer (SAKK 08/08)	II	37	Progression-free survival Progression-free survival was 35 % at 12 weeks	2013	Templeton et al. [135] NCT00976755
The use of RAD001 with docetaxel in the treatment of metastatic, androgen-independent prostate cancer	I–II		No study results reported	2013	NCT00459186
Tasquinimod					
Phase II randomized double blind placebo-controlled study to determine the efficacy of ABR-215050 in asymptomatic patients with metastatic castrate-resistant prostate cancer	II	201	Progression-free survival at 6 months 69 % of subjects receiving tasquinimod were progression-free at 6 months, compared to 37 % in the placebo group ($p<0.001$)	2011	Pili et al. [132] NCT00560482
Dimethylxanthenone acetic acid					
Study of AS1404 with docetaxel in patients with hormone refractory metastatic prostate cancer	II	74	Primary endpoint not specified Median overall survival was 17.0 months for the docetaxel + Dimethylxanthenone acetic acid group, compared to 17.2 months in the docetaxel group	2010	Pili et al. [137] NCT00111618
Enzastaurin					
Phase 2 trial of enzastaurin in prostate cancer in patients who have had hormonal and chemotherapy	II	42	Progression-free survival Median progression-free survival was 11 weeks	2011	Dreicer et al. [140] NCT00428714
PI-88					
Pilot efficacy study of PI-88 with docetaxel to treat prostate cancer	I–II	35	PSA response Trial was stopped early due to toxicity. 70 % had PSA decline >50 %	2010	Khasraw et al. [142] NCT00268593
Cilengitide					
Cilengitide in treating patients with metastatic prostate cancer	II	16	PSA response 0 % had PSA decline of >50 %	2012	Alva et al. [150] NCT00103337
Cilengitide in treating patients with prostate cancer	II	44	Progression-free survival at 6 months 6-month progression-free survival was 9 % in low-dose arm and 23 % in high-dose arm		Bradley et al. [153] NCT00121238
Tanespimycin					
17-AAG in treating patients with metastatic prostate cancer that did not respond to previous hormone therapy	II	15	PSA response No subject achieved a PSA response. Median progression-free survival was 1.8 months	2008	Heath et al. [159] NCT00118092
Retaspimycin					
A phase 2 study to investigate the clinical activity of IPI-504 in patients with hormone-resistant prostate cancer (IPI-504-04)	II	19	PSA response No subject achieved a PSA decline >50 %	2011	Oh et al. [160] NCT00564928

Updated results from this trial have continued to show a PFS advantage to tasquinimod, as well as a non-statistically significant trend towards longer OS [133].

Three additional studies of tasquinimod for prostate cancer are currently underway. A pivotal, placebo-controlled, phase III trial has met accrual and will test tasquinimod monotherapy in patients with chemotherapy-naïve, metastatic CRPC [NCT01234311]. There is also a randomized, double-blinded phase II trial examining tasquinimod as a maintenance therapy for metastatic CRPC in patients successfully treated with docetaxel [NCT00527124]. Finally, a phase I trial of tasquinimod in combination with cabazitaxel is ongoing [NCT01513733]. Based on the promising results of the one study reported so far, data from these trials are highly anticipated.

Everolimus

Everolimus is an inhibitor of mammalian target of rapamycin (mTOR), an intracellular serine-threonine kinase which directs multiple cellular growth and proliferation pathways, making it an agent of great potential benefit. It is currently under investigation for the treatment of multiple malignancies. Everolimus also has anti-angiogenic action as an inhibitor of HIF-1α [134]. Interest in anti-angiogenic effect is increasing, particularly in regard to its potential use in prostate cancer.

Early phase II data for everolimus in CRPC are just beginning to become available, with the first two trials having been reported in early 2013 (Table 15.4). A single-arm, open label trial of everolimus for CRPC enrolled 35 patients, none of whom experienced a biochemical or clinical response [NCT00629525]. Another trial of everolimus monotherapy for chemotherapy-naïve CRPC patients reported that 35 % of 37 patients were progression-free at the pre-specified end point of 12 weeks, though only 5 % (2 of 37) experienced a PSA decline of >50 % [135].

Many additional phase II trials are underway, with several investigating everolimus in combination regimens. Trials currently awaiting results include: a phase II investigating single-agent everolimus for chemotherapy-naïve CRPC [NCT00919035]; a phase II of everolimus in combination with docetaxel and bevacizumab for CRPC [NCT00574769]; a phase II of everolimus plus carboplatin and prednisone for CRPC patients who have previously failed docetaxel [NCT01051570]; a phase I–II of everolimus and docetaxel for CRPC [NCT00459186]; and a phase II of everolimus and bicalutamide for CRPC after failure of first-line androgen-deprivation [NCT00814788].

Dimethylxanthenone Acetic Acid

Dimethylxanthenone acetic acid (5,6 dimethylxanthenone-4-acetic acid), also known as DMXAA, ASA404, and AS1404, is a member of a class of small-molecule "vascular disrupting agents" (VDAs). DMXAA and similar agents work by disrupting the actin cytoskeleton of endothelial cells, producing a rapid collapse of the tumor vasculature that can lead to near-complete shutdown of blood flow within minutes and subsequent tumor necrosis [136].

A single phase II trial has examined DMXAA for use in prostate cancer (Table 15.4). This randomized trial assigned chemotherapy-naïve CRPC patients to docetaxel and DMXAA or docetaxel alone. The DMXAA group saw trends towards improvement in median percentage PSA reduction (84.0 vs. 61.9 %) and radiographic response (23.1 vs. 9.1 %), but these differences were not significant. Two-year survival rates were 33.3 % for the DMXAA group and 22.8 % for the non-DMXAA group, a non-significant difference [137]. Unfortunately, this agent performed poorly in other trials investigating its use for lung cancer, and there are no ongoing efforts to expand upon the early hints of activity seen against CRPC.

Enzastaurin

Enzastaurin (LY317615) is a synthetic bisindolylmaleimide serine-threonine kinase inhibitor targeting protein kinase C (PKC). As PKC is downstream of VEGF within the MAPK signaling pathway, this raises the possibility of anti-angiogenic activity on the part of enzastaurin [138]. Preclinical studies of enzastaurin confirmed this effect, demonstrating a reduction in

tumor vascularity as well as circulating VEGF levels in a mouse tumor model [139].

Only one trial of enzastaurin for prostate cancer has reported results (Table 15.4). In this trial, men with CRPC were grouped according to whether they had received previous treatment with docetaxel, and all were given enzastaurin monotherapy. This trial was stopped early for futility after only one response was observed in the first 18 patients enrolled to the docetaxel-naïve group; upon final analysis, median PFS did not differ from historical controls [140].

PI-88

Phosphomannopentaose sulfate (PI-88) is a sulfated oligosaccharide with structural mimicry of heparan sulfate. PI-88 and related compounds are thought to exert anti-angiogenic effects by preventing recognition of heparan sulfate by angiogenic growth factors, as well as by inhibiting the cleavage of heparan sulfate by heparanase. Preclinical studies have shown a marked reduction in both tumor vascularity and metastatic potential after the introduction of PI-88 to model tumors [141].

A single phase I–II trial of PI-88 for prostate cancer has been undertaken (Table 15.4). After a dose-escalation phase I component, CRPC patients were then randomized to two dosing schedules of PI-88 in combination with docetaxel and prednisone. While the PSA response was encouraging—70 % of patients achieved a reduction of >50 %, more than in historical controls—there was a high level of hematologic toxicity, with 27 % of patients experiencing febrile neutropenia [142]. Evaluation of PI-88 for other solid tumors including hepatocellular carcinoma has continued, but its use in prostate cancer has not been revisited.

TRC105

TCR105 is a monoclonal antibody targeting the CD105 transmembrane receptor. This receptor is involved in TFG-β signaling, and is upregulated in tumor endothelial cells in response to HIF-1α signaling. It is thought to play a primary role in TGF-β-mediated neovascularization, making it a potential target for anti-angiogenic therapy [143]. Preclinical trials have confirmed a significant anti-angiogenic and anti-metastatic effect [144]. Additionally, a phase I first-in-human trial testing TCR105 in multiple solid tumors has reported a favorable side effect profile and a result of stable disease or better in 47 % of 45 patients [143].

Interest in TCR105 is high, and there are currently 12 trials ongoing to study its effects in multiple malignancies, including one phase I–II trial for metastatic CRPC patients who may have received prior anti-angiogenic therapy [NCT01090765] (Table 15.4). Results from these ongoing trials are highly anticipated.

Trebananib

Trebananib (formerly AMG386) is a fusion protein that inhibits the pro-angiogenic factors angiopoietin-1 and -2 from binding to their receptors. Phase I data have shown a favorable side effect profile, with only a small percentage (12 %) of patients experiencing > level 1 toxicity. Additionally, reductions in tumor vascularity and several partial responses were seen in this trial of trabananib for multiple solid tumors [145].

Since then, trabananib has been studied in several randomized, placebo-controlled phase II trials for various malignancies. Results have been disappointing, with trabananib failing to improve PFS or OS when added to other treatment regimens for metastatic renal cell carcinoma [146], gastroesophageal cancer [147], or colorectal cancer [148]. Mixed results were seen with ovarian cancer, with a non-significant trend towards longer PFS [149]. Regarding prostate cancer, trabananib is currently being tested in combination with abiratirone and prednisone for the treatment of metastatic CRPC [NCT01553188] (Table 15.4).

Cilengitide

Cilengitide (formerly EMD121974) is a cyclic pentapeptide that competitively inhibits ligand binding to the $\alpha_v\beta_3$ and $\alpha_v\beta_5$ integrins. $\alpha_v\beta_3$ integrin is expressed in prostate cancer cells but not in the normal prostate. These molecules mediate cell–cell adhesion, particularly within the extracellular matrix of bone tissue, and are

thought to play a central role in the development of bony metastasis; pancreatic cancer cells metastatic to bone uniformly express $\alpha_v\beta_3$. Additionally, integrin-mediated signaling promotes cell survival, apoptosis inhibition, and angiogenesis [150]. *In vitro*, cilengitide has been shown to increase apoptosis of tumor cells [151] as well as inhibit VEGF- and FGF-mediated angiogenesis [152].

Two clinical trials of cilengitide for prostate cancer have been completed—one for metastatic and one for non-metastatic disease (Table 15.4). Non-metastatic CRPC was studied in a single-arm phase II trial of cilengitide monotherapy. With 16 patients enrolled, no PSA responses were observed [150]. Patients with asymptomatic, metastatic CRPC were randomized to two different doses of cilengitide monotherapy; this trial reported a best outcome of stable disease at 6 months, occurring in 9 % of patients receiving the lower dose and 23 % of patients receiving the higher dose [153]. There are no ongoing trials of cilengitide for treatment of CRPC.

Tanespimycin and Retaspimycin

Tanespimycin (17-N-allylamino-17-demethoxygeldanamycin, 17-AAG) is a small-molecule derivative of the antibiotic geldanamycin that acts by inhibiting heat shock protein 90 (Hsp90). Hsp90 promotes multiple functions within the tumor cell, including replication, invasion, apoptosis avoidance, and resistance to hypoxemia via sustained angiogenesis [154]. Furthermore, Hsp90 likely helps tumor cells avoid immune surveillance, as its inhibition has been observed to increase NK cell killing of tumor cells by upregulating MHC class I expression [155].

Phase II trials showed promising results in both breast cancer [156] and multiple myeloma [157]. However, despite these findings, the drug manufacturer (Bristol-Myers Squibb) stopped further development of tanespimycin for reasons that are not entirely understood [158]. One phase II trial of tanespimycin for prostate cancer was completed before drug development was halted (Table 15.4); CRPC patients who had failed previous chemotherapy were given tanespimycin monotherapy. No responses in 15 enrolled patients were observed [159].

After tanespimycin was withdrawn from further study, focus shifted to retaspimycin (IPI-504), a tanespimycin derivative that does not share the parent molecule's hydrophobicity, allowing for better pharmacokinetics and improved drug delivery. One phase II trial of tanespimycin monotherapy for CRPC has been completed, which showed no PSA responses and unacceptable toxicity (Table 15.4); further trials for prostate cancer are not planned [160].

Future Pathways for Angiogenesis in CRPC

The Unmet Potential of Anti-angiogenic Therapy

Despite a solid pathophysiologic rationale founded in our understanding of tumor biology, the clinical results of anti-angiogenic therapies have been disappointing. Though many new agents are still under development or in the nascent stages of clinical evaluation, and further study of existing agents is ongoing, the benefits observed to date of anti-angiogenic therapy for prostate cancer have been modest. Bevacizumab [95], aflibercept [100], lenalidomide [112], and sunitinib [116] have all failed to demonstrate a meaningful benefit when taken to phase III testing. While some newer agents, most notably tasquinimod and cabozantinib, have shown encouraging results in early phase trials, certainly nothing has yet demonstrated an effect of the magnitude that the capability to halt a cancer's vascular supply would seem to promise.

These setbacks should not lead to the conclusion that anti-angiogenesis has no therapeutic role in CRPC. Rather, anti-angiogenesis has failed only as applied in our current treatment paradigm. The failure of this model—the targeting of specific angiogenic pathways by systemically administered drugs in an unselected patient population—was demonstrated well in the VENICE Trial of aflibercept. Based on these results, we can confidently say that the systemic blockade of individual

angiogenic factors, even those as important as VEGF, does not improve outcomes for CRPC patients in aggregate.

However, this is not to say that anti-angiogenic therapies hold no benefit for any patient. Rather, its therapeutic manipulation may require more complexity than the targeting of individual pathways, and more selectivity than attempting to do so in every patient. There are several reasons why the efficacy of these agents may not have been demonstrated in using our current clinical trial design. Suppression of multiple pathways may be necessary. Different tumors may depend on different pathways. Additionally, prostate cancers fall over a broad risk spectrum, and the same benefit might not be seen in treating low-risk disease.

Efforts are already underway to gain a more nuanced understanding of angiogenesis. The future of anti-angiogenic therapy, as well as its promise, may be in knowing not only which pathways to target, but which patients.

The Tumor Microenvironment: Thinking Locally

Our understanding of tumor biology has resulted in an increasing recognition of the microenvironment as an essential factor in determining a tumor's growth and malignant potential. Tumors are no longer thought of as simple, clonal proliferations driven by a single mutated oncogene, but as a complex organ dependent on wide mix of interactions and signals with adjacent cells. Prostate cancers develop in a stromal compartment of fibroblasts, immune cells, endothelial cells, extracellular matrix, and the resulting bath of cytokines, growth factors, and adhesion molecules. Cancerous cells are ultimately dependent on interactions with this stromal compartment to achieve angiogenesis, invasion, and metastasis.

Considerations regarding the interaction, growth, and signaling of tumor cells on the microscopic scale are becoming increasingly relevant to therapeutic strategies. The access to pro-growth signals, extracellular matrix contact, oxygen, etc., may serve to reduce the potency of some treatments and potentiate others via mechanisms that are evident only on the microscale.

The aspect of the prostate cancer microenvironment most directly relevant to angiogenesis, naturally, is the structure of the microvasculature. Rapid cellular proliferation causes hypoxia within tumor tissue, which stimulates hypoxia-inducible factor (HIF) and the resulting pro-angiogenic factors [161]. The new vessels generated within tumors, however, have marked structural abnormalities. They are small and tortuous, branch chaotically, and have abnormal basement membranes. As a result, these vessels are "leaky," allowing for the extravasation of fluid into the intravascular compartment that leads to a localized increased in the interstitial fluid pressure [162].

There is increasing evidence that the microenvironment factors of hypoxia and vascular leakage may directly increase malignant potential by promoting metastasis [163] and causing resistance to both chemotherapy and radiotherapy [164]. Vessel irregularity, which results in vascular leakage, has been identified as a risk factor for developing lethal disease in men with early-stage prostate cancer [38]. Mechanistically, the increased interstitial fluid pressure in the tumor microenvironment decreases the diffusion of intravascular substances, reducing drug delivery [82]. Additional evidence suggests that hypoxia leads to impaired production of oxygen-free radicals, reducing the cytotoxic effect of chemotherapy [165] and radiation [164].

This understanding of microenvironmental factors has led to a new model of how anti-angiogenic therapies might improve prostate cancer treatments: by causing a "normalization" of the abnormal tumor vasculature, and a counter-intuitive *increase* in tissue oxygen delivery [83]. Hypoxia, in a sense, is an adaptive response of the tumor; it leads to the formation of abnormal, permeable vessels that both promote metastasis and protect against chemotherapeutics. Hypoxia also necessitates the use of glycolysis within tumor cells, and the resultant lactic acid contributes to an acidic microenvironment, which limits drug delivery [165]. VEGF inhibitors have been shown to reduce the abnormal histopathology of

tumor blood vessels, producing a decrease in the interstitial fluid pressure and an increase in drug delivery; [166] this is now felt to be an important part of the mechanism of anti-angiogenic therapy.

The tumor microenvironment also provides many new therapeutic targets, with the goal of disrupting the paracrine signals and local biochemistry that promote tumor growth and resilience. The MET oncogene, for example, is also upregulated in response to hypoxia [60], which promotes protease production and loss of cell–cell adhesion, important processes for invasion and metastasis [167]. This provides a conceptual rationale for the dual targeting of angiogenic and MET pathways, such as with cabozantinib, which inhibits both VEGFR-2 and the c-Met receptor [168].

Prostate cancer stem cells rely on interactions with non-malignant cells in the microenvironment. For example, experimental models have demonstrated that fibroblasts present in the tumor stroma promote the proliferation of prostate cancer stem cells [169]. Tumor–stromal interactions therefore provide an additional array of potential therapeutic targets, including Src kinases, the endothelin A receptor ET-1, and TGF-β [168].

As prostate cancer's propensity for bony metastasis plays a large role in its pathology, targeting its ability to thrive in the microenvironment of the bone marrow is another therapeutic possibility. Prostate cancer's successful spread to bone relies on "osteomimicry," a term used to describe the production of normally bone-specific proteins such as bone morphogenic protein, osteocalcin, and bone sialoprotein, by prostate cancer cells [168]. Research is underway on agents designed to disrupt these interactions, including endothelin A receptor and RANK ligand inhibitors. One target of particular interest is the integrin family of cell adhesion molecules. Integrins are involved in a variety of cellular processed, including signal transduction, survival, and proliferation [170]. The α_v-group of integrins, in particular, are up-regulated in human prostate cancer cells [171], where they are responsible for such pathologic functions as bone-homing [172] and angiogenesis [170]. Attempts to prevent prostate cancer cells' ability to manipulate the microenvironment by disrupting integrin signaling are underway; cilengitide, an integrin inhibitor, is already in clinical trials [168], and monoclonal antibodies against integrin are also being developed [172].

An Individualized Approach to Anti-angiogenic Therapy

While some tumors clearly respond dramatically to anti-angiogenic therapy, the majority of clinical trials have failed to observe a substantial effect. The future of anti-angiogenic therapy in prostate cancer, however, may lie in unraveling this seeming paradox. Individual tumors may be driven by vastly different underlying biology; it is only to be expected, then, that applying the same therapy to a broad group of patients will see any effect diluted out by the lack of response in all those patients whose disease is independent of the targeted pathway. An increased ability to decode the biology of individual tumors—whether through quantification of circulating biomarkers, genome sequencing, or other methods—may allow us to create tailored treatment regimens. Furthermore, understanding the risk profile of individual tumors would enhance our ability to target therapy—to identify, for example, a patient with high risk for metastasis who would be more likely to benefit from treatment to achieve "normalization" of the microvasculature.

As an example, certain genetic polymorphisms and biomarkers have already been identified that predict response to bevacizumab. Polymorphisms at VEGF-936 have been reported to influence the response of ovarian cancer to bevacizumab [173]. Preclinical study has found several biomarkers that appear to confer resistance to bevacizumab, including Bv8, PDGF-C, and neuropilin-1 [174]. While none of these findings are in the context of prostate cancer, this malignancy is undoubtedly governed by a similarly complex web of interactions, and further research in this area will surely yield a better understanding of context-specific drug efficacy.

Additional data suggest that the ability of tumors to induce angiogenesis is highly variable, likely due to tumor–host interactions and

microenvironmental factors. Tumors in various tissue sites, for example, require different levels of VEGF in order to produce angiogenesis [175]. Therefore, the plasma VEGF level may be an imprecise measure of a tumor's angiogenic potential [175]. A more detailed understanding of angiogenic signaling and biomarkers of this process may allow us to identify those tumors with the highest risk of developing the disorganized, leaky microvasculature that contributes to metastasis and poor clinical outcomes. Targeting patients with high-risk disease may prove to be a more productive strategy for usage of anti-angiogenic therapy. Identifying biomarkers of response to anti-angiogenic therapy will further improve the appropriate usage of these agents most likely to benefit from therapy [176].

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Co-targeting Adaptive Survival Pathways

16

Amina Zoubeidi and Martin Gleave

Introduction

Androgen deprivation therapy (ADT) is the current first-line systemic therapy for men with advanced prostate cancer (PCa). Unfortunately, ADT provides only a short-term survival benefit due to emergence of lethal castration-resistant prostate cancer (CRPC) [1–3]. Development of this acquired treatment resistance is attributed to an interplay between reactivation of the androgen receptor (AR) axis, along with activation of oncogenic signaling pathways, stress-induced survival genes and cytoprotective molecular chaperone networks, as well as development pathways. There is clear continued dependence on AR signaling during CRPC progression, often indicated by rising serum levels of the AR-regulated prostate specific antigen (PSA). Indeed, frequent responses of CRPC to potent inhibitors of steroidogenesis and AR binding [4] have ushered in the era of second-line hormonal agents using more potent AR-targeting agents in clinical development. These include abiraterone, which targets CYP-17, an enzyme required for de novo intra-tumoral steroidogenesis, and enzalutamide (ENZ), a potent AR antagonist targeting the ligand-binding domain of AR. While response rates with abiraterone and ENZ are encouraging and overall survival is prolonged by 4 months, resistance invariably emerges with rising serum PSA levels, indicating continued AR activity. Hence, while castration and AR pathway inhibitors induce profound and sustained responses in advanced prostate cancer, recurrence is associated with genomic or metabolic reactivation of the AR.

Many anti-cancer treatments activate survival pathways that inhibit apoptosis, contribute to tumor cell plasticity, and promote emergence of an acquired treatment-resistant phenotype. Plasticity refers to a tumor cell adaptation that occurs in response to therapy [5–8], and is a means by which tumor cells escape control by therapy. Blocking tumor cell plasticity may delay or prevent treatment resistance and considerably improve outcomes in advanced prostate cancer. Adaptive pathways are inherent to prostate cancer cells and co-targeting them will create conditional lethality and significantly improve patient outcomes. The concept behind conditional lethality aims to improve therapy by combining targeted therapies under contextualized genetic and environmental conditions that specifically target and kill tumor cells. Adaptive survival pathways triggered by inhibition of a driver mutation, for example AR amplification in the case of CRPC, represent opportunities for conditional lethality and a high therapeutic index. While ongoing direct targeting of the AR will be important, this review will focus on approaches to co-target the AR with cross-talk signaling pathways that

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F. Saad and M.A. Eisenberger (eds.), *Management of Castration Resistant Prostate Cancer*, Current Clinical Urology, DOI 10.1007/978-1-4939-1176-9_16, © Springer Science+Business Media New York 2014

Table 16.1 Adaptive pathways in CRPC

Persistent AR signaling	Stress response pathways	Signaling pathways	Developmental pathways
AR, N-Terminal AR, co-factors LSD-1 and BF3	Hsp27, CLU, autophagy, Hsp90, p38 MAPK	PI3K/Akt, MAPK, JNK, NF-κB, CCL2, MET, src	EMT (N-Cad, Zeb 1,2, Twist, TGF-B, IL-6); WNT, Hedgehog (Shh), MAPK, IL8, Trop-2

cooperatively activate the AR and other survival pathways, or stress response pathways that maintain protein homeostasis and cytoprotection under stress conditions (Table 16.1).

Co-targeting Microtubules with Simultaneous Chemo-Hormonal Combinations

Docetaxel and cabazitaxel are the two taxanes approved for clinical use in CRPC [9, 10]. Docetaxel was the first approved non-hormonal therapy with a demonstrated survival benefit in CRPC. The TAX327 study showed that docetaxel every 3 weeks plus prednisone was superior to weekly mitoxantrone plus prednisone [10]. Median survival in the q3 weekly docetaxel plus prednisone arm was 19.2 versus 16.3 months in the mitoxantrone/prednisone arm [9]. The TROPIC study established the role of cabazitaxel as second-line therapy in CRPC, randomizing men with progressive disease during or after docetaxel to cabazitaxel + prednisone versus mitoxantrone + prednisone [11], and demonstrated 2.4 months improved overall survival with cabazitaxel.

Taxanes function by stabilizing the dynamic polymerization of microtubules. The ability of microtubules to assemble and disassemble is critical for mitosis and thus targeting microtubules preferentially in rapidly dividing cancer cells. Resistance to taxanes may be mediated through overexpression of the multi-drug-resistant P-glycoprotein efflux pump [12], mutations in the microtubule binding sites, and mutations in microtubule-associated proteins giving greater stability to cellular microtubule assembly [13, 14].

Taxanes also affects AR signaling through its alteration of microtubules-associated AR cellular transport and nuclear translocation [15, 16], raising speculation about cross-resistance with AR pathway inhibitors and questions of simultaneous verses sequencing of combinatorial chemo-hormonal regimens. Eigl et al. [17] demonstrated that simultaneous combined taxane-based chemotherapy plus androgen ablation is significantly more effective than the sequential administration of these treatments in the castrate sensitive Shionogi and LNCaP tumor models. Interestingly, a lack of response to castration was observed after initial paclitaxel therapy while gene expression studies confirmed up-regulated of several genes known to play a role in castrate resistance in response to paclitaxel exposure. These findings illustrate how stress-induced gene expression changes after chemotherapy or castration can confer cross-treatment resistance and provide preclinical proof-of-principle for ongoing clinical trials addressing the role and timing of systemic therapies in prostate cancer.

ECOG3805 (CHAARTED) compared standard sequence ADT followed by docetaxel upon castration resistance versus docetaxel given at the start of ADT in 790 men with metastatic castrate sensitive prostate cancer (http://clinicaltrials.gov/show/NCT00309985). Men received either ADT alone or ADT with the chemotherapy drug docetaxel every three weeks over a period of 18 weeks. Men who received simultaneous combinatorial chemo-hormonal lived longer than patients who received ADT alone with 3-year overall survival of 69.0 versus 52.5 %, respectively (nih.gov/news/health/dec2013/nci-05.htm). Patients with a high extent of metastatic disease accounted for most of the benefit in the overall survival from docetaxel plus ADT (3-year survival rates of 63.4 versus 43.9 % for ADT alone). While further follow-up is needed in order to define the effect of this treatment combination on patients with less extensive metastatic disease, this study provides the first example of clinical efficacy using combinatorial therapies in advance prostate cancer and supports the early addition of docetaxel to ADT rather than waiting until CRPC.

Reciprocal Cross-Talk Activation of AKT and MAPK

CRPC is a complex process by which cells survive and proliferate in low circulating androgen. This in part involves the reactivation of the androgen receptor (AR) axis [18], by pro-survival genes and alternative mitogenic growth factor pathways [19–22] including the phosphoinositide 3-kinase (PI3K)/AKT pathway. Indeed, the AR (via amplification) and PI3K/AKT (via Pten loss) pathways are the two most frequently activated signaling pathways in prostate cancer. While AR inhibitors confer clinical responses in most patients, PI3K inhibitors rarely induce tumor regression in preclinical models. Indeed, monotherapy with AR or AKT inhibitors result in reciprocal cross-talk activation that helps emergence of acquired resistance [23–26]. For example, Carver et al. [23] showed that these pathways regulate each other via reciprocal negative feedback, such that inhibition of one activates the other. Inhibition of the PI3K pathway restored AR signaling in PTEN-deficient prostate cells in part through relief of negative feedback to HER kinases; conversely, blockade of AR relieves feedback inhibition of AKT through reduced levels of FKBP5 impairing the stability of the phosphatase PHLPP. Hence while tumor cells could adapt and survive when either single pathway is inhibited pharmacologically, combined inhibition of PI3K/AKT and AR signaling using the PI3K/mTOR inhibitor BEZ235 and the AR antagonist ENZ significantly delayed CRPC progression in the LNCaP model [23].

Thomas et al. [27] reported that monotherapy with the AKT-inhibitor AZD5363 increased AR transcriptional activity and AR-dependent genes such as PSA and NKX3.1 expression. These effects were overcome by the combination of AZD5363 with bicalutamide resulting in synergistic inhibition of cell proliferation and induction of apoptosis in vitro, and prolongation of tumor growth inhibition and PSA stabilization in CRPC in vivo. These studies provide preclinical proof-of-concept that combination co-targeting of an AKT inhibitor with an AR antagonist leads to prolonged delay of CRPC progression.

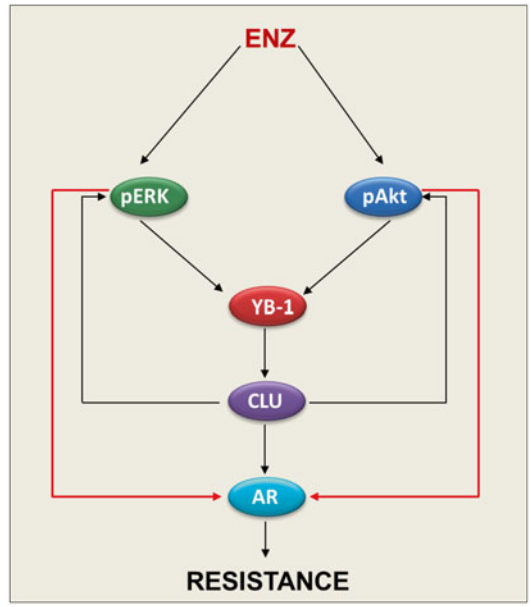

Fig. 16.1 ENZ induces treatment resistance via feed-forward mechanisms involving MAPK and AKT pathways and molecular chaperone CLU

In addition to the AKT pathway, other signaling pathways have been identified that are induced by AR blockade-induced cross-talk activation (Fig. 16.1). For example, AR blockade leads to activation of MAPK pathway which is coordinated by a feed-forward loop involving p90rsk-mediated phospho-activation of YB-1 with subsequently induces the activation of survival pathway leading to the up-regulation of the molecular chaperone CLU, hence inducing treatment resistance [28]. Interestingly, targeting CLU using OGX-011 currently in phase III clinical trials in combination with ENZ not only delay the resistance to ENZ but also inhibit both AKT and MAPK pathways and further provide a preclinical proof-of-principle of co-targeting AR pathway in combination with OGX-011 [28].

Molecular Chaperones and the Stress Response

In cancer, stress is a driving force behind evolution (oncogenesis) and adaptation (acquired treatment resistance). During transformation, tumor cells undergo drastic shifts in their intracellular and

extracellular milieu, frequently exposed to stress microenvironments that include hypoxia, acidosis, nutrient deprivation, and immune attacks from the host. To survive and prosper, like organisms living in the wild, tumor cells must be able to adapt to a variety of stress conditions. Many anti-cancer treatments induce stress responses that inhibit apoptosis and promote emergence of an acquired treatment-resistant phenotype. The heat shock response, for instance, is a highly conserved protective mechanism in eukaryotic cells associated with survival, thermotolerance, and oncogenic transformation [29]. Molecular chaperones play key roles in these stress responses, facilitating treatment resistance by regulating protein homeostasis (proteostasis) as well as many signaling and transcriptional survival networks. Chaperones play central roles in endoplasmic reticular (ER) stress [30–32] and the unfolded protein response (UPR), tailored to re-establish proteostasis by inhibiting translation, increasing chaperone expression, and promoting proteasome- and autophagy-mediated protein degradation. While these adaptive responses are cytoprotective, cell death can occur when ER stress and misfolded protein burden overwhelms the degradation capacity of the proteasome or autophagy [33–38]. Co-targeting stress-induced survival pathways regulating proteostasis, such as ERAD or ERAA, may better manipulate cancer cell sensitivity to therapy.

Molecular chaperones, including heat-shock proteins (Hsps), are key mediators of the stress response, acting as genetic buffer to stabilize and help cells cope at times of environmental stress. During cancer progression, Hsps increase Darwinian fitness of cells during transformation, progression, and treatment resistance [39]. Molecular chaperones bind misfolded proteins to facilitate substrate refolding or degradation, protecting cells against protein aggregation, and facilitating refolding or degradation. Chaperone expression is induced after many varied insults including hypoxia, heat shock, anoxia, glucose deprivation, free radicals, carcinogens, hormone therapy, and chemotherapy [40–43]. Indeed, expression of sHSPs is frequently up-regulated in many cancers and correlates with metastases, poor response to chemotherapy, and poor survival.

The transcriptional regulation of sHSPs is convoluted because of redundancy and feedback control, but several stress-related transcription factors are considered to be major inducers of HSPs. Heat shock factor 1 (HSF-1) is a highly conserved transcription factor that binds to consensus heat shock element (HSE) within the promoter regions of HSP genes [44]. Stress-induction of HSF-1 is a multi-step process that involves constitutive expression of HSF-1 monomer, stress-induced HSF-1 serine phosphorylation, followed by its trimerization, nuclear translocation with increased transcription [45]. HSF1 activates the transcription not only for sHSPs but also other molecular chaperones that associate with HSF1 to initiate a negative-feedback loop and inhibit HSF1 transcriptional activity [46]. Deletion of *Hsf1* gene in mammalian cells does not alter normal basal expression of HSPs but abrogates stress-induced expression of HSPs [47], suggesting that other transcription factors are involved in the basal expression of HSPs. In mice, HSF-1 deficiency inhibits spontaneous tumor formation initiated by dominant negative mutation of p53 and chemical induced skin carcinogenesis associated with activating mutations of H-Ras proto-oncogene [29]. While HSF-1 is not a classic oncogene capable of driving transformation, it does coordinate a broad of network of cellular functions that supports tumorigenesis, highlighting HSF-1 as key regulator of the stress response that is usurped during oncogenesis to promote adaptation and malignant progression.

This section will review roles for Hsp27 and CLU in stress response and acquired treatment resistance, and their current status as therapeutic target in CRPC.

Hsp27

Hsp27 (HspB1) is transcriptionally regulated by HSF-1 and several studies suggest that the specific transactivation of Hsp27 is stimulus dependent [48–50]. The transcriptional regulation of Hsp27 appears to be regulated by many factors influenced by cell type and cell context. Additionally, Hsp27 can be phosphorylated on

three serines (Ser15, ser78, and Ser82) and on Threonine (Thr143). Hsp27 phosphorylation is a reversible process catalyzed by a large number of kinases including MAPKAP kinases 2 and 3, p90Rsk, PKC, PKD, and PKG. Hsp27 becomes phosphorylated in response to a variety of stresses, including oxidative stress, inflammatory cytokines like tumor necrosis factor-α(TNF-α) Interleukin-1β, and transforming growth factor (TGF-β), and mitogens like insulin like growth factor (IGF-1) [51], and steroid hormones [52]. Hsp27 phosphorylation plays an important role in cytoskeleton organization and cell migration. Hence, Hsp27 phosphorylation is required for TGF-β induced metalloproteinase-2 activation and invasion in breast cancer cells and that inhibiting Hsp27 phosphorylation resulted in inhibition of tumor cell migration and invasion [53]. Hsp27 phosphorylation is associated with many malignancies and correlates with p90Rsk in prostate cancer [51] as well as progression to castrate resistance [51].

(a) *Hsp27 regulates cell survival*. Hsp27 is highly and uniformly expressed in treatment-resistant cancers including CRPC [54, 55]. Its expression is induced by hormonal withdrawal and/or chemotherapy, and inhibits treatment-induced apoptosis through multiple mechanisms [56–60]. Hsp27 interacts with and inhibits components of both stress- and receptor-induced apoptotic pathways. It prevents activation of caspases by sequestering cytoplasmic Cytochrome C [61]; interacts with and inhibits Caspase-3 activation; and stabilizes actin microfilaments [62] by binding to F-actin preventing disruption of the cytoskeleton [63]. Hsp27 also regulates Akt and inhibits Bax activation [64]. Hsp27 inhibits apoptosis induced by etoposide or TNF-α in different cancer cell lines by increasing IκBα ubiquitination and degradation, leading to the activation of NF-κB [65]. In AR positive LNCaP prostate cancer cells, androgen stimulates Hsp27 phosphorylation via p38 kinase, which displaces Hsp90 as an AR chaperone and then shuttles AR to the nucleus to facilitate binding to androgen response elements and enhance AR transcription activity and cell survival [52].

After androgen withdrawal, several growth factor pathways play an important role in PCa cell survival, including IGF-1 and IL-6. IGF-1 stimulates Hsp27 phosphorylation in a dose and time dependent manner; Hsp27 is a novel p90Rsk substrate and downstream effector of Erk, increasing IGF-1 induced phosphorylation of Erk, p90Rsk, and Akt [51]. Conversely, Hsp27 knockdown abrogates IGF-1-induced phosphorylation of Erk, p90Rsk and Akt, thereby destabilizing Bad/14-3-3 complexes and increasing apoptotic rates [51]. Hsp27 regulates activity of oncogenic signaling pathways, such as STAT3 [56, 66, 67] which controls expression of cell survival genes and pathways associated with treatment resistance. Hsp27 has a "switch" role in regulating PEA-15 activity to enhance cell proliferation and suppress Fas-induced cell death [68]. Hsp27 enhances Akt activation which, in turn, phosphorylates PEA-15 at Ser-116, switching the binding specificity of PEA-15 from ERK to FADD. The dissociation of PEA-15 from ERK alleviates the cytoplasmic sequestration of ERK, allowing its translocation into the nucleus where it mediates its mitogenic effects. Upon phosphorylation of PEA-15 at Ser-116, PEA-15 binds to FADD and thereby inhibits Fas-induced apoptosis. These findings suggest that patients with tumors harboring abnormalities in PTEN function may clinically respond more favorably to Hsp27 inhibitors [68], thus identifying a population more likely to benefit from Hsp27 inhibition therapy.

(b) *Hsp27 regulates EMT and metastasis*. In addition to its role in cell survival, Hsp27 drives epithelial–mesenchymal-transition (EMT) in PCa, whereas its attenuation reverses EMT and decreases cell migration, invasion, and matrix metalloproteinase activity. Mechanistically, silencing Hsp27 decreased IL-6-dependent STAT3 phosphorylation, nuclear translocation, and STAT3 binding to the Twist promoter, suggesting that Hsp27 is required for IL-6-mediated EMT via modulation of STAT3/Twist signaling. A correlation between Hsp27 and Twist has been observed in patients with prostate

cancer, with Hsp27 and Twist expression each elevated in high-grade prostate cancer tumors. Hsp27 inhibition by OGX-427, an antisense therapy currently in phase II trials, reduced tumor metastasis in a murine model of prostate cancer. More importantly, OGX-427 treatment decreased the number of circulating tumor cells (CTCs) in patients with metastatic castration-resistant prostate cancer in a phase I clinical trial. Overall, this study defines Hsp27 as a critical regulator of IL-6-dependent and IL-6-independent EMT, validating this chaperone as a therapeutic target to treat metastatic prostate cancer. Moreover, it regulates actin rearrangement, cytoskeleton organization and cell migration [53] and thereby enhances cell migration and invasion via modulation of Fak-dependent actin organization and STAT3-dependent MMP-2 expression [69]. Knockdown of Hsp27 inhibits VEGF-induced cell migration [70] and abrogates TGF-β induced MMP-2 as well as cell invasion in human prostate cancer cell lines [71, 72].

(c) *Hsp27 as a prognostic marker*. Clinically, Hsp27 is highly expressed in many cancers including prostate [54, 55] and others [73] and is associated with aggressive tumor behavior, metastasis and poor prognosis [60, 74]. In prostate cancer, expression of Hsp27 in diagnostic biopsy predicts poor clinical outcome [75] and correlates with CRPC progression [56]. Hsp27 expression and phosphorylation correlate with CRPC progression [51, 56]. Hsp27 expression increases shortly after treatment with androgen ablation and becomes highly uniformly expressed in CRPC [51, 54, 56, 74]. Hsp27 expression at diagnosis predicts poor clinical outcome independent of ETS-gene rearrangement [75].

(d) *Hsp27 as a therapeutic target*. As a stress-activated chaperone, Hsp27 expression is induced by hormone and chemotherapy and inhibits treatment-induced apoptosis through multiple mechanisms [51, 56–60]. Consistent with its multiple cytoprotective functions, overexpression of Hsp27 renders human prostate LNCaP tumors resistant to paclitaxel, enhances tumor growth, and confers resistance post castration [56, 76]. Hsp27 interacts with factors involved in oncogenic signaling pathways and CRPC progression such as STAT3, IGF-1, AR, and eIF4E [52, 56, 66, 76]. For example, Hsp27 binds to AR and displaces Hsp90 as the predominant chaperone after androgen treatment, shuttles the AR to the nucleus, and facilitates AR binding to the androgen response element in AR-regulated genes [52]. Additionally, after castration therapy, increased Hsp27 confers resistance by activating STAT3 [56], and by stabilizing eIF4E e- to inhibit its stress-induced ubiquitination and proteasomal degradation to enhance survival [76].

OGX-427 (OncoGeneX Pharmaceuticals) is a second-generation 2′-methoxyethyl phosphorothioate ASO targeting Hsp27 that has a tissue half-life of >7 days. Rocchi et al. first reported ASO-induced Hsp27 knockdown induced apoptosis with single agent tumor growth inhibition as well as enhanced hormone- and chemo-therapy activity when used in combination [74, 77]. Hsp27 inhibition with OGX-427 demonstrated anticancer activity in vitro and in vivo [52, 56, 74, 78], inducing AR [52] and eIF4E [76] proteasomal degradation with decreased AR transactivation and PSA expression in vivo [52]. Hsp27 ASO induces apoptosis and delays prostate tumor progression [74] and chemo-sensitizes bladder, prostate, ovarian, and uterine cancer to paclitaxel [74, 79, 80], and inhibits metastasis dissemination in murine model of prostate cancer [67]. Since Hsp27 functions as a regulatory "hub" in multiple adaptive survival signaling and transcriptional pathways, it is an attractive therapeutic target; Hsp27 inhibition may simultaneously suppress many pathways implicated in cancer progression and resistance to hormone- and chemo-therapies.

OGX-427 has completed single agent and docetaxel-combination dose escalation phase I trials in prostate, bladder, breast, and lung cancer. OGX-427 is well tolerated at 800 and 1,000 mg dose in combination with docetaxel. Reduction in tumor markers was observed in patients with prostate (PSA) and ovarian (CA-125) cancer. Decline of 50 % or greater in both total and

Hsp27+ CTCs was observed in over half of the patients [67]. A randomized phase II study of OGX-427 plus prednisone versus prednisone alone in patients with chemotherapy-naive metastatic castration-resistant prostate cancer (CRPC) has also been completed. Patients with CRPC and no prior treatment were enrolled 1:1 to receive prednisone 5 mg PO BID alone or with OGX-427 600 mg IV × 3 loading doses in week 1 followed weekly 2-h infusions on a 4 week cycle. Preliminary results indicated that 82 % of patients treated with OGX + prednisone had a PSA decline (45 % with >30 % decline) and 18 % a PSA increase compared to 50 % of patients treated with prednisone have had a PSA decline (20 % with >30 % decline) and 50 % a PSA increase [81]. CTC conversion from ≥5 to <5/7.5 ml occurred in 50 % of patients treated with OGX + prednisone and 20 % of patients treated with prednisone alone. These results confirm, for the first time, single agent activity for an Hsp27 inhibitor in cancer, and phase II combination studies are ongoing in CRPC, lung, pancreas, and bladder cancer.

Clusterin

Secretory CLU (CLU) is a multifunctional, stress-induced, ATP-independent molecular chaperone containing amphipathic and coiled-coil helices in addition to large intrinsic disordered regions. These properties of CLU resemble survival chaperones associated with tissue injury and pathology like acute phase protein haptoglobin [82] and small Hsp's [83]. Indeed, CLU is involved in many biological processes ranging from mammary and prostate gland involution to amyloidosis and neurodegenerative disease, as well as cancer progression and treatment resistance [84]. Promoter sequences of *CLU* gene are conserved during evolution and include stress-associated sites like activator-protein-1 (AP-1), AP-2, SP-1, HSE (heat shock element) recognized by HSF-1/HSF-2 heterocomplexes, and CRE (cAMP response element) [85]. Additionally, there are glucocorticoid response element (GRE) [86–88], androgen response element (ARE) [89], and Y-box binding protein (YB-1) sites [90]. CLU expression increases downstream of survival signaling pathways and in response to ER-stress. CLU is up-regulated downstream IGF-1 via Src-Mek-Erk-EGR-1 [91] and cytokines via Jak/STAT1 [92] and downstream of ER stress inducer including paclitaxel via YB-1. YB-1 directly binds to CLU promoter regions to transcriptionally regulate clusterin expression. In response to endoplasmic reticulum stress inducers, including paclitaxel, YB-1 is translocated to the nucleus to transactivate CLU. YB-1 transactivation of CLU in response to stress is a critical mediator of paclitaxel resistance in prostate cancer [90].

CLU Enhances Cell Survival Under Stress Conditions

sCLU functions to protect cancer cells from many varied therapeutic stressors that induce apoptosis, including androgen or estrogen withdrawal, radiation, chemotherapy, and biologic agents [93]. CLU is regulated by HSF1 [85], and functions like small HSPs to chaperone and stabilize conformations of proteins at times of cell stress, potently inhibiting stress-induced protein precipitation by binding to exposed regions of hydrophobicity on non-native proteins to form soluble, high molecular mass complexes [94–96]. CLU inhibits ER stress, retro-translocating from the ER to the cytosol to inhibit aggregation of intracellular proteins and prevent apoptosis [97]. Interestingly, CLU is the most abundant protein associated with β-amyloid deposits in Alzheimers, likely related to its role in inhibiting protein aggregation [94]. Collectively, the preceding indicates sCLU plays an important role in protein homeostasis (proteostasis) via unfolded protein and ER stress responses.

CLU inhibits mitochondrial apoptosis by suppressing p53-activating stress signals and stabilizes cytosolic Ku70-Bax protein complex to inhibit Bax activation [98], interacting with conformationally altered Bax to inhibit apoptosis in response to chemotherapeutic drugs [99]. In addition, CLU increases Akt phosphorylation levels and cell survival rates [100] and promotes prostate cancer cell survival by increasing NF-κB

nuclear transactivation, acting as a ubiquitin-binding protein that enhances COMMD1 and I-kB proteasomal degradation via interaction with E3 ligase family members [101]. Experimental and clinical studies associate CLU with development treatment resistance, where CLU suppresses treatment-induced cell death in response to androgen withdrawal, chemotherapy, or radiation [102–104].

CLU Correlates with Adverse Prognosis

CLU is expressed in many human cancers, including breast, lung, bladder, kidney, colorectal, and prostate [105–109]. In prostate, CLU was originally cloned as "testosterone-repressed prostate message 2" (TRPM-2) [110] from regressing rat prostate, but was later defined as a stress-activated and apoptosis-associated, rather than an androgen-repressed, gene [102]. CLU expression correlates with loss of the tumor suppressor gene Nkx3.1 during the initial stages of prostate tumorigenesis in Nkx3.1 knockout mice [111]. High levels of CLU expression associate with migration, invasion and metastasis, increasing Smad2/3 stability and enhancing TGF-β-mediated Smad transcriptional activity [112]. In contrast, CLU silencing induces mesenchymal–epithelial-transition via inhibition of Slug [113].

CLU levels are low in low-grade cancers, but increase with higher Gleason score [109]. Levels of sCLU increased several fold after androgen ablation, consistent with its stress-activated, cytoprotective response to anti-cancer treatment [104]. Biochemical recurrence-free survival in patients with strong CLU expression in prostatectomy specimens was lower than those with weak CLU expression [114]. Plasma levels of sCLU were significantly higher in patients with high-grade prostate cancer with extra-capsular extension compared to organ-confined tumors [115]. These data correlate CLU with higher grade, post treatment stress, and/or poor outcome in many cancers.

CLU as Therapeutic Target

CLU levels are high in CRPC [102, 103] and after estrogen withdrawal in breast cancer cells [116–119], cisplatin [120, 121], doxorubicin [122, 123], Herceptin [124], Hsp90 inhibitors [125], and HDAC inhibitors [126]. In keeping with its cytoprotective function, CLU inhibition enhances cytotoxicity of hormone- radiation-, and chemo-therapies [119, 127, 128]. The antisense inhibitor, OGX-011 (custirsen), is a second-generation ASO with a long tissue half-life of ~7 days that potently suppresses CLU levels in vitro and in vivo. OGX-011 improved the efficacy of many varied anti-cancer therapies by suppressing treatment-induced CLU and the stress response [125] preclinical activity in many xenograft models of cancer [128–131]. CLU ASO sensitizes bladder cancer to cisplatin [121], prostate to paclitaxel [103], and prostate to hormone therapy [102] where it delays CRPC progression [121, 102, 125, 132].

The first-in-human phase I study with OGX-011 used a novel neoadjuvant design to identify effective biologic dosing of OGX-011 to inhibit sCLU expression in human cancer [133]. Neoadjuvant androgen deprivation was administered concurrently. At doses of 320 mg and higher, concentrations of full-length OGX-011 were achieved that were associated with preclinical activity. OGX-011 produced statistically significant, dose-dependent >90 % knockdown of CLU in normal and tumor tissue and identified 640 mg as the optimal biologic dose for Phase II trials. Plasma pharmacokinetic parameters have been similar across phase I studies including when OGX-011 was combined with chemotherapy and decreases in serum sCLU have been consistently observed [134, 135]. A phase II trial of 85 patients with non-small cell lung cancer (NSCLC) treated with combined OGX-011 and gemcitabine-cisplatin chemotherapy [135, 136] reported an objective response rate of 23 % and median overall survival of 383 days with 58 % surviving >1 year.

A randomized phase II study in chemo-naïve, metastatic CRPC randomized 81 patients to either docetaxel-OGX-011 or docetaxel-alone [137]. The median cycles delivered for docetaxel-OGX-011 was 9 compared to 7 for docetaxel-alone. There was evidence of biologic effect with 18 % decrease in mean serum sCLU in patients treated with docetaxel-OGX-011 versus 8 % increase in controls ($P = 0.0005$). Median overall

survival on the docetaxel- OGX-011 arm was 23.8 months, 7 months longer than those receiving docetaxel-alone (16.9 months) (HR = 0.49, $P = 0.012$) [138]. Given the survival outcomes observed in the docetaxel-OGX-011 arm, randomized phase 3 studies have been initiated. Another trial of docetaxel-recurrent CRPC randomized 42 patients to receive either docetaxel or mitoxantrone combined with OGX-011, to test whether OGX-011 could reverse docetaxel resistance or improve mitoxantrone efficacy in a chemo-resistant population [139, 140]. PSA declines of ≥30 % were seen in 55 % of docetaxel-OGX-011 patients and 32 % of mitoxantrone-OGX-011 patients. Pain responses were also seen in >50 % of patients and after a median follow-up of 13.3 months, 60 % of patients were alive in both arms. These results are also of interest considering PSA response rates of <20 % and median survival <12 months is usually reported in patients with docetaxel-resistant CRPC receiving second-line chemotherapy [141], supporting further studies second-line indications for CRPC. Currently, three Phase III studies are ongoing, co-targeting CLU-regulation of chemotherapy induced-stress with OGX-011 in combination with chemotherapy in CRPC and lung cancer. For example, SYNERGY has enrolled over 1,000 men with chemo-naïve metastatic CRPC randomized to docetaxel-alone or plus OGX-011, and will readout in 2014.

Autophagy

Many physiologic stressors are closely linked to autophagy activation, an evolutionarily conserved process designed to degrade long-lived proteins and organelles to maintain protein and metabolic homeostasis [142, 143]. During autophagy, macromolecules or organelles are sequestered in autophagosomes, which fuse with lysosomes to degrade protein aggregates and provide recycled building blocks for anabolism and energetics [144]. Autophagy helps cells adapt to proteotoxic, metabolic and other stress by catabolizing misfolded proteins or damaged structures to maintain homeostasis.

The relationship between autophagy and cancer is complex and contextual. Early in carcinogenesis, autophagy is tumor-suppressive, reducing accumulation of damaged proteins or organelles and genomic damage under stress conditions [145, 146]; indeed, defective autophagy can lead to tumor development [147]. However, in established cancers autophagy is cytoprotective, particularly under stress conditions, facilitating cell survival and adaptation by eliminating toxic protein aggregates and providing sources of nutrients to maintain protein and metabolic homeostasis [145, 146, 148, 149].

Under unstressed conditions, the PI3K/Akt pathway inhibits autophagy through the activation of mTOR; under nutrient, growth factor deprived, or hypoxic conditions, autophagy is activated by the AMPK pathway, leading to up-regulation of autophagy genes and suppression of mTOR/S6K/4EBP activity by phosphorylation of TSC2 [150]. Autophagy biogenesis is multi-step process characterized by the induction, nucleation, extension, and completion of an isolation membrane phagophore, regulated by about 30 autophagy-related (Atg) genes including 2 ubiquitination-like conjugation steps crucial to the formation of autophagosome membranes. The autophagosome then fuses with lysosomes where contents are degraded into amino acid, fatty acid and nucleotides to be reused.

Cancer cells use autophagy to prolong their survival under harsh conditions of metabolic stress induced by chemotherapy, ionizing radiation, nutritional starvation, oxidative stress or growth factor deprivation to facilitate acquired treatment resistance [151–156]. Indeed, modulating autophagy has recently exploited as a molecular target to improve cancer therapy and accumulating evidence showed that targeting autophagy alone or in combination with chemotherapy enhances cell death and efficacy of cancer therapies many cancers. In preclinical cancer models, inhibition of autophagy can enhance chemosensitivity and tumor cell death [156–159]. Using prostate cancer models, Lamoureux et al. [160] reported that the Akt inhibitor AZD5363 reduced p-mTOR and p-S6K, induced G(2) growth arrest and autophagy, but failed to induce

apoptosis. Blocking autophagy using pharmacologic inhibitors (3-methyladenine, chloroquine) or genetic inhibitors (siRNA targeting Atg3 and Atg7) enhanced AZD5363-induced apoptosis and the combination of AZD5363 with chloroquine significantly reduced tumor growth compared with the AKT-inhibitor monotherapy [160].

Autophagy was recently identified as a survival mechanism that prostate cancer cells used to overcome AR pathway inhibition. AR knockdown [161], androgen deprivation, or bicalutamide treatment induced autophagy, while autophagy inhibition sensitized LNCaP cells to bicalutamide-induced apoptosis [162]. Inhibiting autophagy can also overcome resistance to ENZ therapy in CRPC. In-vivo studies with mice orthotopically implanted with ENZ-resistant cells demonstrated that the combination of ENZ and autophagy modulators, clomipramine (CMI) or metformin significantly reduced tumor growth [163].

Currently, the only FDA approved agents that inhibit autophagy are chloroquine, an antimalarial drug, and its derivative hydroxychloroquine (HCQ). Several clinical studies using the autophagy inhibitor, hydroxychloroquine, are underway [164–166]**.** One completed clinical trials using HCQ suggested a survival advantage when adding to conventional treatment for glioblastoma multiforme but the result was not statistically significant [166]. Additional agents with autophagy-inhibitory actions include clomipramine and metformin. These data support autophagy as an adaptive response to AR pathway inhibition and that pharmacological-inhibition of autophagy can impair prostate cancer cell survival, identifying a potential role in combinatorial AR co-targeting strategies.

Co-targeting Adaptive Survival Pathways

While new drugs like docetaxel, ENZ, abiraterone, and others have re-shaped the treatment landscape for CRPC, an ongoing major clinical challenge is how to integrate these into optimal sequencing and combinatorial regimens to delay the emergence of drug-resistant tumors. In the face of redundant pathways and the heterogeneous characteristics of cancer, it is not surprising that one specific pathway has not been identified as the primary cause of drug resistance in CRPC and therapies targeting single pathways have limited benefits. Investigating combination therapies targeting molecules involved in cross-talk of multiple signaling pathways that induce conditional lethality is needed. For example, targeting AR using ENZ induces cellular stress involving activation of Akt [23] and MAPK which is coordinated by a feed-forward loop involving p90rsk-mediated phospho-activation of YB-1 with subsequent induction of CLU [28]. CLU knockdown in combination with ENZ accelerates AR degradation and repressed AR transcriptional activity through mechanisms involving decreased YB-1 regulated expression of the AR co-chaperone, FKBP52. Co-targeting the AR (with ENZ) and CLU (with OGX-011) synergistically enhanced apoptotic rates over that seen with ENZ or OGX-011 monotherapy and delayed CRPC LNCaP tumor and PSA progression in vivo [28]. Moreover, Hsp90 inhibitors increase CLU levels both in vitro and in vivo. Silencing CLU using siRNA or OGX-011 abrogates Hsp90 inhibitors induced HSF-1 transcriptional activity, while CLU overexpression enhances, Hsp90 inhibitor-induced HSF-1 transcription activity, identifying a role for CLU in the regulation of HSF-1 and the heat shock response itself [125]. CLU knockdown blocks the translocation to HSF-1 to the nucleus following treatment with Hsp90 inhibitors. This effect of CLU on HSF-1 activity is biologically relevant since CLU overexpression protects, while CLU silencing enhances, cytotoxicity of Hsp90 inhibitors in vitro, while OGX-011 synergistically enhanced Hsp90 inhibitor activity in vivo in PC-3 and LNCaP models by enhancing treatment-induced apoptosis. Collectively, these results highlight, for the first time, a biologically relevant feed-forward regulation loop of CLU on HSF-1 and the heat shock response [125].

Hsp27 is known to protect prostate cancer cells against proteotoxic stress induced by proteasome inhibition; indeed, Hsp27 silencing using antisense

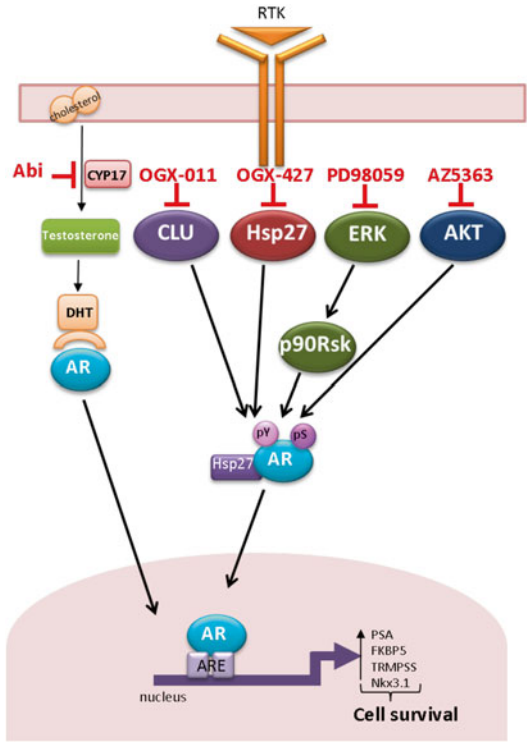

Fig. 16.2 Common signaling pathways responsible for AR activation in ENZ resistance that can be co-targeted to achieve conditional lethality

(OGX-427) induced both apoptosis and autophagy through mechanisms involving reduced proteasome activity and induction of endoplasmic reticulum (ER) stress. These findings identify autophagy as a cytoprotective, stress-induced adaptive pathway, activated following disruption of protein homeostasis and ER stress induced by Hsp27 silencing [167]. Preclinical data has shown that co-targeting Hsp27 and autophagy by combining OGX-427 with the autophagy inhibitor, chloroquine, significantly delayed PC-3 prostate tumor growth in vivo.

These data highlight how co-targeting adaptive stress pathways activated by AR pathway inhibitors or cytotoxic agent are mediated through MAPK, AKT, CLU, or Hsp27 (Fig. 16.2), creates conditional lethality and provides mechanistic and preclinical proof-of-principle to guide biologically rational combinatorial clinical trial design.

Conclusion

In summary, defining key mediators of treatment stress and adaptive survival pathways will enable co-targeting strategies that create conditional lethality and improve outcomes. Prioritization of pathways to target must be guided by robust preclinical proof of principle package, contextually relevance, and clear understanding of mechanistic interactions between multi-targeted pathways. Leading examples include combination strategies of AR antagonists with inhibitors of AKT, Hsp27, CLU, or autophagy that collectively regulate cytoprotective transcriptional and signaling pathways involved in acquired treatment resistance.

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Targeting the PI3K/AKT/mTOR Pathway in Prostate Cancer

17

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The PI3K/AKT/mTOR pathway is one of the most frequently altered pathways in human cancers. Regardless its activation, this pathway has been linked to cell survival, differentiation, proliferation, growth, metabolism, migration, and angiogenesis. In normal cells, signaling through this pathway begins typically with binding of a growth factor to a receptor tyrosine kinase molecule resulting in downstream activation of phosphatidylinositol 3-kinase (PI3K). Three classes of PI3Ks have been described. The Class IA PI3Ks are the most relevant from an oncologic perspective and consist of a regulatory subunit p85 and a catalytic subunit p110. The gene encoding the alpha isoform of the p110 subunit, PIK3CA, is frequently mutated in cancer. Three isoforms of the Class IA p 85 regulatory subunit are encoded by the PIK3R1-3 genes. Alternatively, activation of PI3K can occur via Ras signaling, mediated by G-protein-coupled receptors. Class IA PI3Ks phosphorylate their substrate, phosphatidylinositol 4,5-bisphosphate (PIP2) to produce phosphatidylinositol 3,4,5-triphosphate (PIP3). PIP3 can subsequently bind to the pleckstrin homology (PH)-domains of various signaling proteins, induce their membranous localization, and initiate downstream signaling primarily via the AKT protein (protein kinase B). This pathway is directly opposed by the protein tyrosine phosphatase, PTEN, which dephosphorylates PIP3 to PIP2 thereby terminating further downstream signaling [1, 2]. The PI3K/AKT signaling cascade promotes cell survival and resistance to apoptosis through several different mechanisms, including interactions with the Bcl-2 family members BAD and BAX, NF-κB, and the p53 antagonist Mdm2. Downstream of this pathway is the mammalian target of rapamycin (mTOR) complex. Activation of mTOR leads to increased protein synthesis through phosphorylation of ribosomal proteins and translation elongation factors. In this fundamental way, mTOR is an important modulator of cell growth and differentiation. Multiple feedback loops and regulators control mTOR signaling, and this pathway integrates inputs from various metabolic, growth factor, and survival pathways [3].

Laboratory data has provided a compelling foundation for studying the role of inhibitors of PI3K and its downstream targets in prostate cancer. Taylor et al. [4] performed genomic profiling of 218 primary and metastatic prostate cancers, integrating information gathered from assessment of DNA copy number, mRNA expression profiles, and focused exon sequencing. A core pathway analysis showed that altered signaling in the PI3K pathway was present in nearly half of all primary prostate tumors and virtually all prostate cancer metastases tested. Approximately 40 % of all cases demonstrated loss-of-function of PTEN through deletion, silencing mutation, or reduced expression. In contrast to

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F. Saad and M.A. Eisenberger (eds.), *Management of Castration Resistant Prostate Cancer*, Current Clinical Urology, DOI 10.1007/978-1-4939-1176-9_17,

Table 17.1 Selected ongoing clinical trials of drugs targeting the PI3K/AKT/mTOR pathway in prostate cancer

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

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

many other cancers (such as breast cancer), activating mutations in the *PIK3CA* gene itself were rare. However, loss-of-function mutations in the regulatory subunits *PIK3R1* and *PIK3R3* were prevalent, suggesting another mechanism for constitutive activation of PI3K in prostate cancer [4].

Despite these important laboratory observations, attempts to target segments of the PI3K/AKT/mTOR signaling pathway in prostate cancer patients have been somewhat disappointing thus far, at least when these agents have been examined as monotherapies. To this end, studies of the mTOR inhibitors rapamycin, everolimus, and temsirolimus when used as single-agents (and even when combined with androgen receptor antagonists, such as bicalutamide) failed to demonstrate significant clinical activity in metastatic castration-resistant prostate cancer (CRPC) patients [5–7]. Nevertheless, based on preclinical data that mTOR inhibition can reverse chemotherapy resistance in PTEN-deficient prostate cancer cell lines [8], ongoing trials are now examining the efficacy of combined treatment with mTOR inhibitors and docetaxel chemotherapy [9, 10]. Other novel mTOR inhibitors and combination therapies are also under investigation (summarized in Table 17.1).

One possible explanation for the inability of single-agent mTOR inhibitors to show efficacy in prostate cancer is the hypothesis that mTOR blockade leads to feedback-driven upregulation of signaling molecules upstream in the PI3K pathway. For example, rapamycin and rapalogs are primarily inhibitors of mTORC1 (mTOR in complex with raptor) but not mTORC2 (mTOR in complex with raptor). This might lead to compensatory phosphorylation of S473, one of the

activation sites of AKT. Thus, there is a theoretical advantage of utilizing active site inhibitors of mTOR [11]. Seminal research by Carver et al. [12] has demonstrated the existence of bidirectional cross-talk between the PI3K pathway and androgen receptor (AR) signaling. For example, in a preclinical model, inhibition of the PI3K pathway resulted in activation of AR signaling in PTEN-deficient prostate cancer cells. Conversely, the AR antagonist enzalutamide appeared to upregulate AKT signaling by reducing levels of the regulatory phosphatase PHLPP. Moreover, combined blockade with the dual PI3K/mTOR inhibitor, BEZ235, administered together with enzalutamide led to reductions in tumor size in xenograft models of human prostate cancer that exceeded the effects seen with either agent used alone [12]. This work provides a sound rationale for simultaneous targeting of the androgen/AR pathway and the PI3K/mTOR pathway, a discovery that is beginning to be translated into the clinic (Table 17.1).

As a case in point, the dual PI3K/mTOR inhibitor BEZ235 [13] is currently being studied in combination with abiraterone in men with metastatic CRPC. The first-in-human phase I study of BEZ235 showed that this agent (used as a monotherapy) was tolerable, and no dose-limiting toxicities were observed at the doses tested. Frequently reported adverse events included fatigue and gastrointestinal symptoms. A few tumor responses were seen in this phase I study, which enrolled patients with various solid malignancies including advanced CRPC. Perhaps not surprisingly, patients whose tumors demonstrated activated PI3K pathway signaling were the most likely to benefit from treatment with BEZ235 [14]. Because of pharmacokinetic variability, the drug was reformulated to improve bioavailability, which delayed clinical development of this agent. However, multiple phase I and phase I/II studies are now underway to investigate the role of BEZ235 in CRPC. The majority of these studies are focusing on dual inhibition of the androgen/AR and PI3K/mTOR pathways (Table 17.1).

Efforts to develop the potent and specific AKT inhibitor, MK2206, are also seeking to capitalize on the preclinical observations that simultaneous AR blockade and PI3K pathway inhibition may be synergistic. Prior phase II studies of an early putative inhibitor of AKT, perifosine, have been disappointing [15, 16]. However, correlative pharmacodynamic studies were not performed in perifosine-treated patients, and therefore it is unclear whether target inhibition was actually achieved at the tumor level. Conversely, pharmacodynamic correlates to the phase I study that established the safety profile and maximum-tolerated-dose of MK2206 has confirmed its ability to target and inhibit AKT in humans [17]. The most frequent side effects of MK2206 observed in this phase I study were hyperglycemia, nausea, and diarrhea. The commonest dose-limiting toxicities were skin rash and stomatitis. It remains to be seen whether MK2206 will prove to be more efficacious than perifosine in the clinical arena. Encouragingly, MK2206 is currently being investigated in conjunction with bicalutamide in a cooperative group trial enrolling men with PSA-recurrent (non-metastatic) prostate after failure of local therapy. Patients in this trial are receiving therapy with bicalutamide alone or combined with MK2206 (Table 17.1).

Finally, the pan-PI3K inhibitors, BKM120 and PX-866, are also being tested in phase II trials of metastatic CRPC. Both of these agents potently inhibit wild-type as well as mutant class-I PI3K isoforms. Phase I studies have included only a handful of patients with prostate cancer, although one man with metastatic CRPC that received PX-866 experienced prolonged stable disease [18]. Interestingly, although the two drugs purport the same mechanism of action, their side effect profiles are somewhat distinct. Dose-limiting toxicities on the PX-866 phase I study were primarily gastrointestinal, including diarrhea and transaminitis. In the phase I study of BKM120, similar gastrointestinal symptoms were seen but the drug had additional toxicities including rash, hyperglycemia, and neuropsychiatric effects such as mood alterations and depression [19]. As phase II trials with these two agents move forward (Table 17.1), attention to the correlative pharmacodynamic studies will be imperative.

In conclusion, targeting the PI3K/AKT/mTOR pathway appears to be a rational approach in prostate cancer, especially in view of the frequent overactivation of this pathway in advanced CRPC. However, the use of mTOR pathway inhibitors as monotherapies is unlikely to bear fruit, due to negative feedback-induced activation of different nodes within the same pathway or stimulation of other reciprocal pathways such as androgen/AR signaling. The most promising future approach will therefore probably rely on simultaneous inhibition of the mTOR pathway and the androgen/AR pathway, although large definitive trials testing this hypothesis are still several years away. Finally, all phase I and II studies targeting this pathway moving forward must incorporate tumor material to establish target inhibition (and even reciprocal signaling effects) in treated patients.

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PARP Inhibitors

18

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PARP and DNA Repair

Cells are continuously exposed to noxious agents causing damage of genetic material, either due to external effectors (toxins, radiations) or internal stresses as a result of physiological processes. DNA repair systems are set to protect the genome, either by amending the damage in a particular cell or initiating programmed cell death if the damage cannot be repaired. In general, disruption of such DNA repair capacities and accumulation of genome aberrations predispose to tumorigenesis.

Diverse systems of DNA repair have been described to liaise with specific types of genome damage: for DNA single strand breaks (SSB), where the complementary DNA strand is intact and serves as template, the base excision repair (BER) or the mismatch repair (MMR) pathways are activated; on the other hand, the homologous recombination (HR; in dividing cells) system or the non-homologous end joining (NHEJ; for cells in G0 stage of the cell cycle) pathways would operate in the event of DNA double strand breaks (DSB) [1]. As will be discussed in this chapter, BRCA1/BRCA2 genes encode proteins that are essential for HR-mediated DNA repair, which is the preferred system over NHEJ for DSB repair, as it is error-free and therefore does not lead to genomic instability.

Poly(ADP-ribose) polymerases (PARP) represent a family of enzymes encoded by different genes with a common catalytic domain involved in the post-translational modification of different proteins. They catalyze the covalent polymerization of ADP-riboses on the aspartic or glutamic acid residues of other proteins using NAD^+ as a substrate, creating long linear and branched poly(ADP-ribose) (PAR) chains [2]. Among this family of enzymes, PARP-1 and PARP-2 have a role in the cellular response to DNA damage. Although their functions do not completely overlap, there is some degree of redundancy. PARP-1 is the most abundant enzyme of the PARP family and also has a role in transcription regulation. It is composed of three major domains: a DNA-binding domain, an automodification domain, and a catalytic domain [3, 4]. PARP-1 and PARP-2 are activated in the presence of DNA breaks, leading to the recruitment of effectors that facilitate DNA repair, and suppressing the inappropriate recombination of homologous DNA due to its presence in the replication fork [5]. PARP inhibitors compete with the natural ligand of the enzyme, NAD^+, to bind PARP and thereby impeding its function. Additionally, some PARP inhibitors can adhere to, and trap, PARP–DNA complexes [6].

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PARP-1 and PARP-2 have DNA-binding domains and are involved in DNA repair processes. These DNA-binding domains localize PARP-1 and PARP-2 to the site of DNA damage, and serve as genome damage sensors. They also facilitate signaling for other molecules to approach the replication fork to repair the DNA break. Despite the more widely described role of PARP in SSB repair, knowledge of this field continues to evolve, and the involvement of PARP enzymes with other mechanisms of DNA repair, including both SSB and DSB, is now recognized [7, 8].

PARP Inhibition as a Therapeutical Tool in Cancer

In 2005, Farmer and colleagues reported data from preclinical experiments laying down the foundations for the therapeutic exploitation of PARP inhibition in cancer medicine [9]. It was shown that silencing of PARP-1 with siRNA drove BRCA1/2 deficient cell lines toward apoptosis. Next, exquisite antitumor sensitivity of the BRCA1/2 deficient cell lines to PARP inhibition was observed, while similar effects were not observed in both BRCA1/2 heterozygous and BRCA1/2 wildtype cells. In addition, they demonstrated that silencing of BRCA1/2 induced sensitivity to PARP inhibition. These key findings provided the preclinical rationale that supported a synthetic lethal approach with PARP inhibitors in patients with BRCA1/2 carrier cancers. At the same time, Bryant and co-workers presented data confirming that PARP inhibition leads to γH2AX and RAD51 foci formation, as well as selective antitumor effects on *BRCA2* mutant cells, in contrast to BRCA wildtype cells [10].

The reason for this selectivity is due to complementary effects of PARP and BRCA proteins on DNA repair systems. When SSB occur, PARP enzymes initiate the repair process; if these PARP enzymes are suppressed (e.g., when exposed to a PARP inhibitor drug), the SSB are converted to DSB at the site of the replication fork. If the mechanisms of DSB repair are conserved, the cell should still be able to repair the damage. However, if the DSB repair systems are also impaired, as in the case of BRCA1/2-deficient cells, the DSB will remain and eventually lead to the initiation of a cascade of events resulting in cell death. Therefore, a cell is still viable in the presence of either of the two defects, but if both PARP and BRCA functions are suppressed simultaneously, the cell will not be able to repair the damage efficiently, and inevitably undergoes cell death. This principle is known as "synthetic lethality" (Fig. 18.1); it is a concept that has been described in biology for more than 60 years since Dobzhansky first published his studies on the variability of different populations of Drosophila [11]. PARP inhibitors now represent a novel application of synthetic lethality, especially as a therapeutic approach in cancer medicine. Such a strategy exploits *BRCA1/2* mutations as an oncogenic advantage for tumor cells, by increasing cell susceptibility to PARP inhibition. Importantly, since *BRCA1/2* germline mutation carriers will conserve one intact copy of the affected *BRCA1/2* gene in normal cells, while cancer cells remain homozygous for the mutation, treatment with PARP inhibitors should have a selective antitumor effect on cancer cells since normal cells will still be able to repair DNA damage; this provides a wide therapeutic window for selectively treating such patients with minimal drug-associated toxicities. Preclinical studies indicate that cells with deficiencies in other proteins involved in HR DNA repair such as ATM, ATR, CHEK2 or RAD51 also result in PARP inhibitor sensitivity resulting in what has been described as BRCA*ness* [12].

Several PARP inhibitors are at different stages of clinical drug development, basically exploring antitumor activity in patients harboring genetic aberrations in HR-mediated DNA repair. An alternative approach is to take advantage of the capacity of PARP inhibitors to potentiate the effects of DNA-damaging agents, such as certain chemotherapeutics or radiation, by disabling the capacity of the tumor cell to repair the damage induced by platinum drugs or radiotherapy, thereby impacting on the survival of such cells. Several preclinical studies have demonstrated

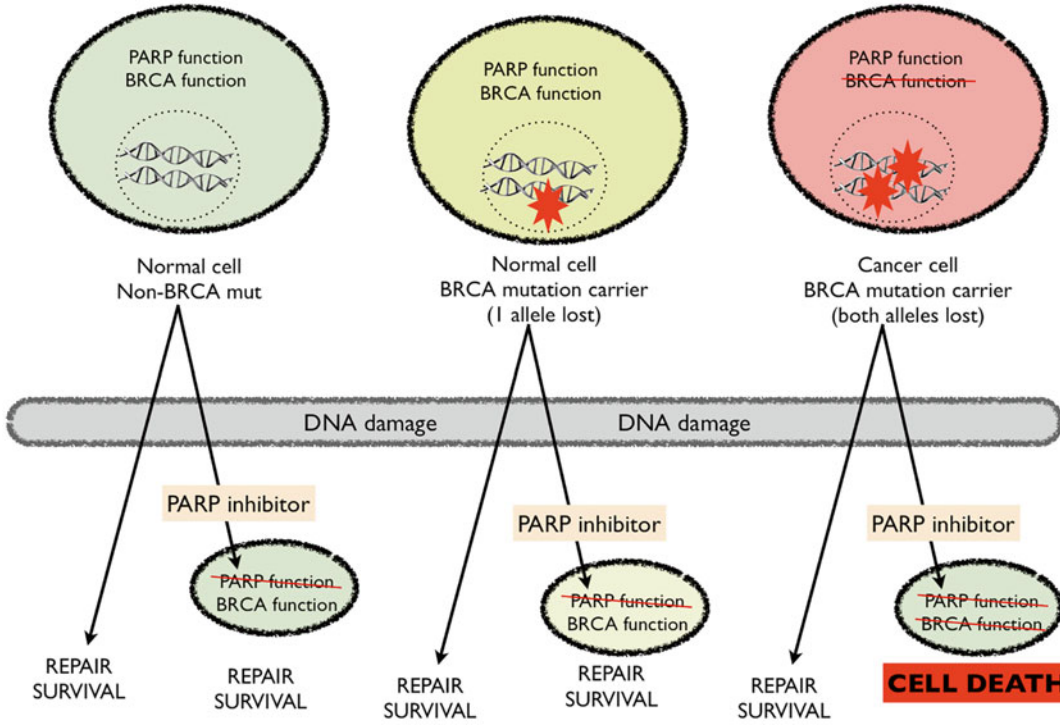

Fig. 18.1 The principle of synthetic lethality applied to cancer medicine. While the loss of either PARP or BRCA function independently does not compromise the ability of the cell to repair DNA damage, PARP inhibition in a cell which has lost BRCA function (*right*) results in inability to repair the damage and consequently, cellular death

how combination regimens with PARP inhibitors sensitize tumor cells to platinums, anthracyclines, alkylating agents, and topoisomerase inhibitors [13, 14]. PARP is a major effector of DNA strand break repair following damage induced by radiotherapy treatments, which may lead to the development of therapy resistance. There is therefore strong rationale to assess PARP inhibitors as radiosensitizer.

Moreover, aside from its main role in DNA repair there is evidence that PARP-1 is involved in the transcription regulation of the androgen receptor (AR) and in the rearrangement of specific genes, such as ERG, ETV1, and FLI1 [16–18]. Studies demonstrating robust preclinical antitumor activity in models expressing TMPRSS2-ERG rearrangements, together with evidence of the suppression of AR-targeted gene expression, support the targeting of PARP in prostate cancer [15–17].

The Target Population for PARP Inhibitors

Although loss of a variety of genes involved in HR DNA repair may sensitize to PARP inhibition, to date the only predictive biomarker of response to PARP inhibitors that has been validated in clinical trials is the presence of germline mutations in the *BRCA1/2* genes.

BRCA1 and *BRCA2* are both tumor suppressor genes coordinating mechanisms of response to DNA damage through HR-mediated DNA repair. While BRCA2 function seems to be limited to DNA repair control, generally through the regulation of RAD51, BRCA1 may have an additional range of functions, with effects over cellular control systems, chromatin modeling, and the regulation of several transcription factors [18–20].

BRCA1/2 (germline) mutation carriers with prostate cancer currently undergo treatment similar to patients with sporadic prostate cancer. This is despite the fact that prostate cancer in *BRCA* mutation carriers has a more aggressive phenotype. They usually present with a higher Gleason score, develop nodal and metastatic involvement more frequently, and have worst survival outcomes [21, 22]. Nevertheless, their rates of response to taxane-based chemotherapy are not dissimilar to the general population [23]. The question as to whether such patients harbor a genetically defined subset of prostate cancer that is responsive to platinum-based chemotherapy remains to be elucidated.

BRCA2 mutations confer an 8.6-fold increase in the risk of developing prostate cancer in men up to 65 years of age, while *BRCA1* mutations increase the risk by 3.5-fold [24, 25]. In animal models, *BRCA2* dysfunction has been shown to induce the appearance of premalignant prostate lesions, but other genetic events are believed to be necessary for carcinogenesis [26]. For example, it is hypothesized that genomic instability secondary to DNA repair impairment paves the way for other oncogenic events, including those present in prostate cancer.

Interestingly, since the BRCA protein acts also as a regulator of the AR pathway, there is increasing interest in combining DNA repair-targeting agents with AR-axis directed therapies. Similarly, crosstalk between the phosphatidylinositol 3-kinase (PI3K)–AKT pathway and BRCA1 provides a strong rationale for the investigation of combination regimens of PARP inhibitors and drugs targeting the PI3K–AKT signaling network [27–29].

Based on strong preclinical rationale demonstrating tumor-specific antitumor activity of PARP inhibitors in *BRCA1/2* mutant cells [9, 10], a phase I proof-of-concept study of olaparib (KU-0059436; AZD2281; AstraZeneca) was commenced. The study population was initially enriched with germline BRCA mutation carriers, but following the observation of objective antitumor responses in this subgroup of patients, recruitment in the phase I dose expansion cohorts was limited to this genetically defined population [30].

Since then, a number of clinical trials have tested the antitumor efficacy of olaparib and other PARP inhibitors both in germline *BRCA1/2* mutation carriers and sporadic cancer populations [31–35]; Gelmon and colleagues reported a clinical trial on olaparib for patients with advanced ovarian or triple negative breast cancers, stratifying them on the basis of the presence of germline *BRCA1/2* mutations [36]. Among sporadic (*BRCA1/2* wildtype) patients, there were no objective responses in patients with breast cancer. However, patient benefit was observed in those with platinum-sensitive ovarian cancer, due to the induction of DNA damage. The expression of certain DNA repair markers, which may be impaired in *BRCA1/2* mutant cells, is associated with increased sensitivity to platinum-based chemotherapy [37].

Germline mutations in *BRCA1/2* genes are inherited in an autosomal dominant manner, with incomplete penetrance, and are present in less than 2 % of sporadic prostate cancers [25, 38]. Therefore, the target population that is likely to have greatest potential to benefit from PARP inhibitors may be small, in comparison with triple negative breast cancer where the prevalence of *BRCA1/2* mutations ranges between 10 and 20 % in unselected populations [39, 40]. There is therefore great interest in finding other potential response biomarkers of response to PARP inhibition through studies investigating the DNA repair system. Evidence of clinical activity in patients who are not *BRCA1/2 mutation carriers*, such as sporadic high grade ovarian cancer and sporadic CRPC supports a wider role for PARP inhibitors [36, 41]. Theoretically, the application of the concept of synthetic lethality to PARP inhibition would not only be limited to *BRCA1/2* germline mutation carriers, but also include patients with functional loss of DNA repair capacity through alternative mechanisms [42]. Importantly, *BRCA1/2* mutations only account for a fraction of known defects in HR-mediated DNA repair. Sensitivity to PARP inhibitors in preclinical models with genetic and epigenetic aberrations involved in the response to DNA damage has led to an armamentarium of potential markers that now need to be evaluated in the clinical setting

including: (a) the phosphatase and tensin homolog (PTEN) gene not only through its role as a negative regulator of PI3K but as a warrant of chromosomal integrity and regulator of RAD51 transcription [43]; (b) tumors with other DNA repair genomic aberrations including deficiencies in ATM, ATR, CHEK2, RAD51, PALB2, and other FANC genes [12, 44]; and (c) epigenetic alterations silencing wild-type *BRCA1/2* and other DNA repair genes [45]. The critical interaction between PARP1, DNA-PKc and the resulting protein of gene fusions, especially of the androgen-responsive gene transmembrane protease serine 2 (*TMPRSS2*) with the oncogenic erythroblast transformation specific (*ETS*) transcription factor family of genes, present in approximately 50 % of prostate cancers, has generated great interest in evaluating this recurrent fusion protein in prostate as a potential biomarker of sensitivity to DNA repair targeting [16].

In order to optimize the application of PARP inhibitors, there is an urgent need to develop and validate clinical biomarkers that interrogate the functionality of HR-mediated DNA repair systems. Such assays may use gene expression and/or protein transcription profiles to identify tumors not known to harbor *BRCA1/2* mutations, but which express similar biological features, a phenotype coined as a "BRCAness" profile. A potential approach is the use of large short-interfering RNA (siRNA) panels to screen potential predictors of PARP inhibitor sensitivity [46].

It is critical that clinical trials evaluating PARP inhibitors in prostate cancer implement functional assays so as to characterize their pharmacodynamic effects and aid in the identification of predictive biomarkers of PARP blockade. Such strategies may evaluate the formation of RAD51 and γH2AX foci in tumor and surrogate tissue, including circulating tumor cells, peripheral blood mononuclear cells, and hair follicles.

Clinical Experience with PARP Inhibitors

The proof-of-concept phase I study of olaparib prospectively enriched each dose escalation with patients harboring germline *BRCA1/2* mutations before restricting accrual to patients with BRCA1/2 mutant tumors in the dose expansion phase [30]. The study identified fatigue, mood alteration, somnolence, and thrombocytopenia as dose-limiting toxicities. There were no significant differences in the toxicity profile between *BRCA1/2* mutation carriers compared to WT *BRCA1/2* patients. Establishment of the biologically active dose-range of olaparib was guided by parallel pharmacokinetic and pharmacodynamic evaluation of normal tissue, including peripheral blood mononuclear cells from blood, hair follicles, and tumor tissue samples. The dose selected from this dose-escalation study was 400 mg twice daily and although 100 mg was demonstrated to have biologically relevant effects, subsequent studies have shown a dose–response relationship for olaparib efficacy. The higher dose of 400 mg BD has therefore been utilized as the preferred dose for later-stage studies [33, 35].

Significant evidence of antitumor activity was observed in this phase I trial among *BRCA1/2* mutations carriers, as predicted from preclinical data [9]. There was evidence of antitumor activity in germline BRCA1/2 mutation carriers suffering from CRPC, including a patient who had a response lasting for almost 3 years, including complete radiological resolution of bony metastases and continued prostate specific antigen (PSA) tumor marker response (Fig. 18.2).

Several phase II studies of olaparib have now been pursued; overall, the compound has been shown to induce substantial antitumor activity as a single agent in *BRCA1/2* mutation carriers with ovarian and breast cancer, as well as several cases of sporadic ovarian cancer. In this later tumor type, analysis of a series of *BRCA1/2* mutation carriers demonstrated cross-sensitivity between prior platinum therapies and PARP inhibitors [34]. Conversely, patients who develop disease progression on PARP inhibitors have been found to still have the potential to respond to further lines of platinum-based chemotherapy [47].

Pivotal studies evaluating the role of olaparib at different stages of ovarian cancer and other tumor types are ongoing or planned, either as monotherapy or in combination with other antitumor agents. There is also an ongoing two-stage phase II study evaluating the antitumor activity of

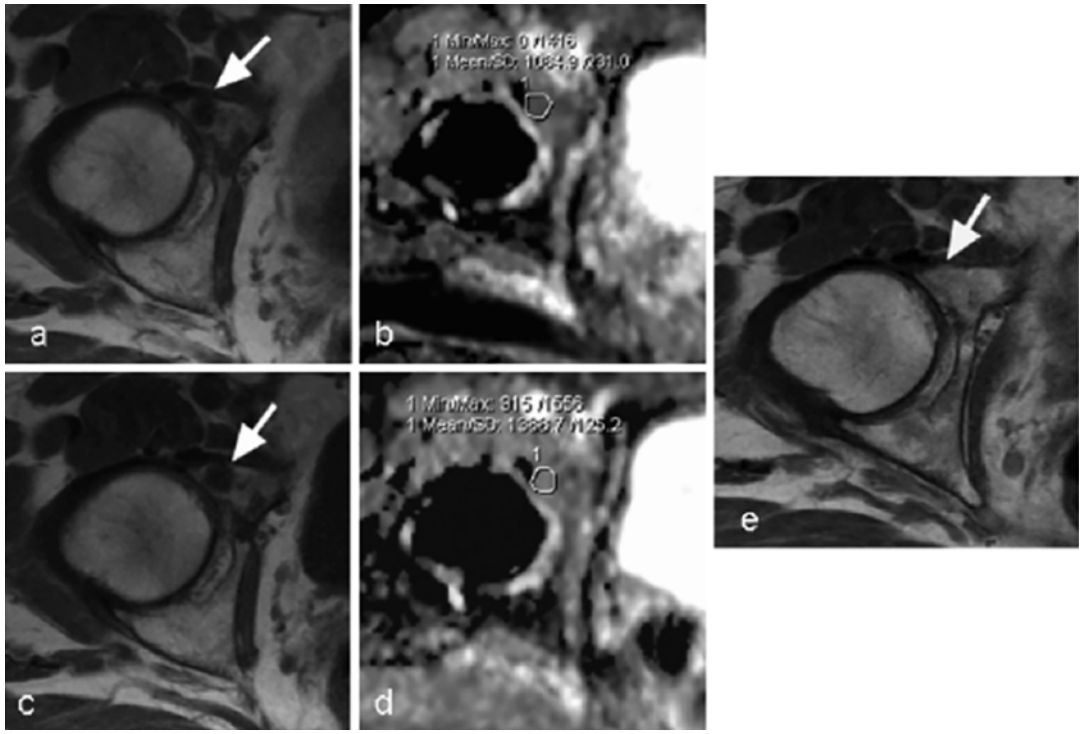

Fig. 18.2 Diffusion-weighted magnetic resonance imaging (MRI) demonstrating disease regression in a patient with prostate cancer and BRCA2 mutation associated with a >50 % decline in PSA. A 63-year-old man with germline BRCA2 mutation and castration-resistant prostate cancer. T1 weighted MRI at the level of the right acetabulum in the pelvis obtained (**a**) prior to and (**c**) 3 months after initiating treatment with olaparib showed no substantial change in a 13 mm low-signal intensity (*dark*) metastasis in the *right* acetabulum (*arrows*). Apparent diffusion coefficient values increased from pre-treatment (**b**) to after 3 months of treatment (**d**) >30 % at the site of metastatic disease (*circled*) consistent with disease regression. (**e**) T1-weighted imaged 1 year after starting treatment showing resolution of disease (*arrow*). Images courtesy of Dr. Dow-Mu Koh, The Royal Marsden NHS Foundation Trust, UK. Adapted from Fong et al., NEJM 2009

olaparib in unselected patients with advanced CRPC. This study aims to identify predictive biomarkers to guide appropriate patient selection that would be validated in a subsequent cohort of patients (NCT01682772) (Fig. 18.3).

Veliparib, previously known as ABT-888 (Abott Laboratories), was shown to inhibit PARP-1 and PARP-2 in preclinical models and to potentiate the effects of DNA-damaging agents, such as cytotoxics or radiation [13, 48]. An initial phase 0 trial was conducted, comprising the administration of single doses of veliparib to patients with advanced cancers, followed by the assessment of pharmacokinetics and pharmacodynamics through subsequent tumor biopsies and surrogate tissue analysis [49]. This aided in the design of dose-escalation trials of veliparib in combination with different chemotherapies. Among the numerous combinatory trials in different tumor types, a phase II study is assessing the combination of veliparib with abiraterone acetate in patients with CRPC (NCT01576172). A separate study treated 25 CRPC patients with veliparib and temozolomide; of these patients, one had a 37 % decrease in PSA and another had a 97 % decrease in PSA associated with a radiological response [50].

The phase I trial of niraparib (MK-4827; Merck, Tesaro) enrolled 18 patients with sporadic CRPC in an expansion cohort at the maximum tolerated dose (300 mg QD) and three further treated during dose escalation. This study

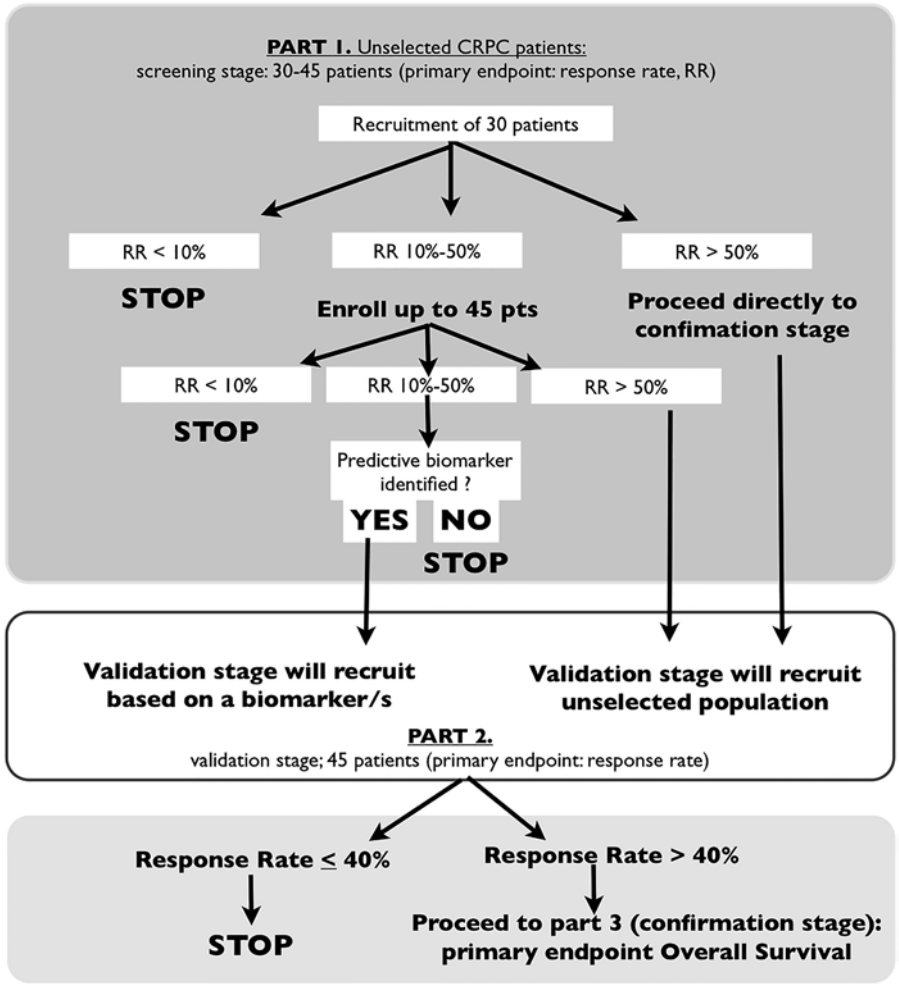

Fig. 18.3 Adaptative design of the TOPARP study, an open-label phase II study of olaparib in CRPC patients to evaluated antitumor activity and investigate predictive biomarkers

recruited a total of 60 patients with advanced solid tumors in the dose-escalation stage, including 29 patients with *BRCA1/2* mutations; the latter group of patients included 22 with advanced ovarian or primary peritoneal cancer, four patients suffering from breast cancer and individual cases of pancreatic, lung, and prostate cancers. A second stage of the study recruited a further 22 patients with sporadic ovarian carcinoma and the aforementioned 18 sporadic CRPC patients, on the basis of the relevance of DNA repair defects in the pathogenesis of these diseases [41].

Pharmacodynamic studies showed that PARP inhibition in peripheral blood mononuclear cells exceeded 50 % at most doses greater than 80 mg/day. Induction of γH2AX foci in circulating tumor cells and a limited number of paired tumor biopsies was demonstrated. 8/20 (40 %) and 2/4 (50 %) *BRCA1/2* mutation carriers with ovarian and breast cancer, respectively, achieved radiological partial responses. Antitumor radiological and/or biochemical responses were detected in 5 of the 22 patients with sporadic ovarian cancer, predominantly in those with platinum-sensitive disease.

Among the 21 patients with CRPC, nine had radiological stability for more than 4 months, with a median duration of treatment of 254 days in this subgroup. One patient experienced >50 %

decrease in PSA on treatment, remaining on treatment for 10 months. Three patients had significant declines in CTC counts, which were maintained for at least 8 months prior to disease progression. Interestingly, one of the three patients participating in the dose-escalation phase of the study was a *BRCA1/2* mutation carrier who did not benefit from niraparib. This study included several biomarker-finding studies for PARP inhibitors in prostate cancer, including the assessment of PTEN function and the presence of ERG fusions in archival tumor samples and circulating tumor cells from 18 CRPC patients in the study. No correlation between PTEN and ERG status with decreases of PSA or time to tumor progression was found.

Rucaparib (AG-014699/CO-338, Pfizer/Clovis Oncology) is another oral PARP1/2 inhibitor that has been assessed in two dose-escalation studies; one as monotherapy and another in combination with several chemotherapy regimens in a population enriched with but not limited to *BRCA1/2* mutation carriers [51, 52]. In preclinical studies, rucaparib induced selective cytotoxicity in tumor cells that were defective in HR-mediated DNA repair [53]. The recruitment of patients with sporadic cancers is supported by preclinical studies demonstrating enhancement of chemotherapy effects in different ovarian cancer cell lines with alternative gene aberrations related with DNA repair mechanisms, such as loss of PTEN function, low expression of RAD51, or silencing by methylation of wildtype BRCA [54]. An intravenous (IV) formulation had previously been evaluated in a dose escalation study in patients with *BRCA1/2* mutant ovarian and breast cancers. Preliminary clinical studies reported an overall response rate of 5 % at dose levels evaluated, which are lower than equivalent oral doses investigated. The IV formulation was also evaluated in combination with chemotherapy, such as with temozolamide in a clinical trial in patients with advanced melanoma [55].

Preliminary results of a first-in-human study on the PARP inhibitor BMN-673 (BioMarin) were reported at the 2013 ASCO Annual Meeting [56]. BMN-673 demonstrated high potency in inhibiting PARP in preclinical studies and showed antitumor cytotoxicity in cells with deficient *BRCA1/2* or PTEN function [57]. Overall, initial reports of the trial showed good oral bioavailability for BMN-673, which induced tumor responses in patients with germline *BRCA1/2* mutant breast and ovarian cancers. Hematological events were dose-limiting, resulting in the selection of 1,000 mcg QD as the dose for further development, which was tenfold above the minimum dose to show target modulation.

As PARP inhibitors are implemented in clinical practice, our understanding of the underlying mechanisms of both primary and secondary resistance will be even more relevant and critical for the optimal application of this class of drugs. A common feature to all molecular targeted agents developed over the past decade is the inevitable development of drug resistance, mostly through tumor evolution in the context of selection pressures induced by prolonged drug exposure. For PARP inhibitors, mechanisms of resistance may include expulsive pumps that decrease intracellular drug availability, a progressive reliance of the cells on alternative mechanisms of DNA repair, and restoration of BRCA1/2 function through gene reversion [58, 59].

Tolerability and Side Effects

Based on the concept of synthetic lethality, the effects of PARP inhibitors are expected to be tumor-specific, with minimal impact on normal cells, which are heterozygous for *BRCA1/2* mutations and therefore expected to conserve DNA repair capacities. DLTs identified during early phase trials of olaparib were: grade 3 mood alteration and fatigue on the first day of a patient receiving 400 mg BID, grade 3 somnolence with 600 mg BID, and grade 4 thrombocytopenia 600 mg bd [30]. Importantly, all DLTs were reversible. The most important side effects described included nausea, vomiting, and fatigue, which are usually mild and manageable with supportive medication. Further studies of olaparib in larger populations of patients with breast and ovarian cancer have shown similar patterns of toxicities, with higher incidence of

myelosuppression noted at higher doses; the overall incidence of grade 3–4 hematological events is 10–15 % [31, 33, 35].

Thrombocytopenia also limited dose-escalation in the phase I study of BMN-673, with cases of grade 2–3 anemia described at higher doses. Fatigue was the most commonly observed toxicity reported (30 %), but most cases were grade 1 (12/21 patients), and only 1 case of grade 3 fatigue was reported [56].

DLTs reported in the phase I trial of niraparib [41]—which recommended a phase II dose of 300 mg QD—included grade 3 fatigue (1/6 patients receiving 30 mg qd), grade 3 pneumonitis (1/7 patients receiving 60 mg qd), and grade 4 thrombocytopenia (2/6 patients receiving 400 mg qd). Further episodes of grade 3–4 hematological toxicities were detected at higher doses of niraparib after continuous exposure for several cycles. Overall, 33 % of patients who started niraparib at the recommended phase II dose required a dose reduction during treatment due to hematological toxicities. No cases of neutropenia were associated with fever in this study. As an indirect comparison with a treatment recently approved for prostate cancer, the registration trial of cabazitaxel chemotherapy administered to patients following the failure of previous docetaxel therapy reported a 82 % rate of grade 3 neutropenia with 8 % of patients experiencing febrile neutropenia [60]. It is remarkable that the toxicities reported from these different PARP inhibitor studies were similar, irrespective of the *BRCA1/2* mutation status of the patients.

The exact mechanisms by which PARP inhibition causes myelosuppression are not completely understood, but it is hypothesized that PARP1 regulates the expression of some hematological growth factors, such as erythropoietin [61]. Concerns have been raised regarding the risk of myelodysplasia after chronic exposure to PARP inhibitors, and thus long-term follow up data will be important. A better understanding of the potential risks of long-time exposures to PARP inhibitors is necessary to pursue the evaluation of these compounds as maintenance therapy after cytotoxic drugs, and even more in strategies of chemoprevention in patients with *BRCA1/2* mutations.

As discussed, another strategy for the development of PARP inhibitors is to combine them with chemotherapies or radiation. If PARP inhibition impairs the capacity of the cell to repair DNA damage induced by cytotoxics or radiotherapy, it may potentially have additive or synergistic effects leading to cell death. However, potentiation of possible side effects from chemotherapy or radiation may also ensue. Clinical trials of different combinations of chemotherapy with PARP inhibitors such as rucaparib, olaparib, or veliparib have reported potentiation of hematological toxicities, often limiting the delivery of standard doses of chemotherapy [52, 62–64]. Indeed, selection of the optimal dose and schedule of PARP inhibitors and the partner cytotoxic when aiming to potentiate the effects of chemotherapy is a challenge that needs to be addressed in specific dose-finding trials for each individual combination.

The antitumor agent iniparib (BSI-201; BiPar Sciences, Sanofi-Aventis), which was initially evaluated in clinical trials as a "PARP inhibitor" in advanced triple negative breast cancer patients [65, 66], showed no significant myelosuppression, or enhancement of chemotherapy-mediated hematological toxicities. Subsequent preclinical studies confirmed that iniparib was indeed not an inhibitor of PARP [67, 68]. As such, any conclusions from iniparib studies should not be extrapolated to the family of PARP inhibitors.

Conclusions and Future Directions

In conclusion, several compounds inhibiting PARP have demonstrated promising antitumor activity with a good tolerability profile and wide therapeutic index; the most common drug-related toxicities observed thus far include myelosuppression and fatigue. The development of this class of antitumor drugs has generally followed a rational biological strategy, based on the concept of synthetic lethality. To date, the only validated predictive biomarker of response is the *BRCA1/2* status. The response rates among this population have been impressive, including patients with advanced CRPC. Although long-term survival

data are yet to be clarified, several PARP inhibitors are currently being evaluated in pivotal trials to define their optimal place in the treatment of advanced ovarian and breast cancers.

Development of specific trials in *BRCA1/2* mutation carriers with CRPC has been challenging due to the low prevalence of this mutation in unselected populations of such patients. It may thus be pertinent to centralize the care of such individuals in nominated institutions, or to conduct studies in specific geographical areas where the prevalence is higher. Considering the underlying mechanism of action for PARP inhibitors, it is expected that other biomarkers of response, mainly along the DNA repair pathway, could be identified. Efforts from both academic investigators and pharmaceutical industry also now need to focus on conducting studies to identify and validate such potential predictive biomarkers in the clinical setting, with the aim of establishing a wider role for these drugs in sporadic cancers.

Moreover, PARP inhibitors have also been shown to generate antitumor activity through the potentiation of certain chemotherapies and radiation, but optimization of combinatorial therapies is challenging due to the enhancement of toxicities. The biological interactions and reciprocal regulation of the AR pathway, PI3K–AKT–mTOR signaling network and DNA repair systems support the conduct of preclinical and clinical studies evaluating the combination of PARP inhibitors with androgen axis-targeting drugs, including abiraterone or enzalutamide and with inhibitors of the PI3K–AKT–mTOR pathway.

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Targeting C-Met/VEGF in Castration Resistant Prostate Cancer

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MET Expression and Role in Prostate Cancer

c-Met is a proto-oncogene that encodes Hepatocyte Growth Factor Receptor (HGFR/MET), a receptor tyrosine kinase expressed in epithelial and endothelial cells [1–3]. Under normal circumstances, MET is activated by HGF produced by stromal cells, such as fibroblasts, thus generating a paracrine activation loop. Phosphorylation of MET triggers the activation of downstream signaling pathways, including the Ras-mitogen-activated protein kinase (MAPK) pathway and the phosphoinositide 3-kinase (PI3K)–AKT pathway through the adaptor proteins Gab-1 and Growth Factor Receptor Binding Protein 2 (GFRBP2) [4]. Non-receptor Src tyrosine kinase has also been described as a downstream target molecule in MET signaling [5]. The HGF/MET pathway is essential for embryogenesis and tissue homeostasis [6]. However in carcinogenesis, the HGF/MET axis has been related to the regulation of the metastatic process [7]. In vitro studies have shown that HGF can function as a mitogen, activating invasive cell growth, proliferation, branching morphogenesis, migration, invasion, as well as angiogenesis [8–10]. MET abnormalities have been described in human cancers. MET mutation resulting in aberrant ligand-independent signaling has been described in papillary renal cell carcinoma and head and neck cancer [11, 12].

In prostate cancer, MET activation appears to be mainly dependent on the ligand, via a paracrine model [13]. MET has been found to be more highly expressed in prostate carcinoma compared to benign prostate hyperplasia, and correlates with higher tumor histologic grade [14–16]. Urinary MET level has been associated with prostate cancer metastasis [16]. MET over-expression is an independent predictor of invasion and metastasis in prostate cancer, appearing to be more common in bone metastases [13, 17]. Moreover, increased serum HGF level is an independent prognostic marker in patients with advanced disease stage [18, 19]. Androgen suppression results in HGF up-regulation and activation of MET in human prostate cancer cell lines, with a transition from paracrine to autocrine signaling, resulting in androgen-independent growth. MET expression was shown to be inversely correlated with AR expression in prostate cancer cell lines, with androgen withdrawal resulting in induction of c-Met expression [20, 21]. Collectively, the

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F. Saad and M.A. Eisenberger (eds.), *Management of Castration Resistant Prostate Cancer*, Current Clinical Urology, DOI 10.1007/978-1-4939-1176-9_19,

HGF/MET pathway appears to play a critical role in the development of resistance to androgen suppression in prostate cancer, and thus has emerged as an appropriate candidate for targeted therapies in CRPC.

Role of VEGF/VEGFR and Cross-Talk with HGF/MET Pathway in Prostate Cancer

Vascular endothelial growth factor (VEGF) signaling is crucial for angiogenesis, a critical step for tumor growth. In comparison to normal prostate tissue and high-grade prostatic intraepithelial neoplasia (PIN), prostate malignant tissue exhibits significantly higher micro-vascular density, which correlates with higher tumor grade and pathologic stage. VEGFR-2 expression appears to correlate with high-grade disease, while VEGF is expressed in the neovasculature of prostate cancer tissue, but not in benign prostatic epithelial tissue [22, 23]. Patients with metastatic prostate cancer have higher plasma VEGF levels, and VEGF plasma or urine levels are negative independent predictors of overall survival (OS) in metastatic CRPC [24–26]. VEGF may also regulate the epithelial to mesenchymal transition (EMT) of prostate cancer cells [27–29]. VEGF promotes the osteoblastic activity of prostate cancer cells despite the fact that prostate cancer cells per se may not express receptors for VEGF (VEGFR1, VEGFR2) [30, 31]. VEGF contributes to tumor-induced bone remodeling at metastatic sites. VEGF phosphorylates MET, and thus activates the HGF/MET pathway through its co-receptor neuropilin-1 (NRP1) which is highly expressed in prostate cancer cells [32]. Overexpression of NRP1 and activation of MET are associated with progression and bone metastases in human prostate cancer specimens and xenografts. MET activation by NRP1 appears to maintain the expression of the anti-apoptotic protein Mcl-1, in prostate carcinoma cells. In these cells, MET inhibition using small interfering RNA (siRNA) attenuated the VEGF-induced Mcl-1 expression [32].

Preclinical Data on HGF/MET and VEGF/VEGFR Pathway Inhibition in Prostate Cancer

Inhibition of MET signaling pathway by a variety of methods reduces both the development and progression of prostate cancer metastases [33, 34]. BMS-777607, a small molecule MET inhibitor suppresses HGF-stimulated cell scattering, migration, invasion, MET auto-phosphorylation, and downstream signaling through the PI3K/AKT/mTOR and MAPK pathways in vitro [35]. Two other small molecule MET inhibitors, PHA-665752 and PF-2341066, result in decreased cell proliferation of both AR-sensitive and AR-resistant prostate cancer cells, but the effect is more potent in AR-resistant cells [36]. PF-2341066 also induces prostate tumor growth suppression in mouse models of prostate cancer with greater effect after castration [36]. Cabozantinib (XL184), a small molecule that inhibits multiple receptor tyrosine kinases, including MET, VEGFR2 and RET, rapidly induces apoptosis of endothelial cells and tumor cells, and inhibits the progression of osteolytic and osteoblastic lesions in xenograft models [37, 38]. Bevacizumab, a humanized monoclonal antibody against VEGF suppresses cell proliferation, angiogenesis, and invasion in the bone-metastatic C4-2B prostate cancer cell line [39]. In vitro and in vivo data suggest that sunitinib, an inhibitor of multiple receptor kinases, including VEGFR and PDGFR, is active against prostate cancer models with intact PTEN expression or concurrent PI3K/AKT/mTOR inhibition [40]. Overall, these preclinical data provide the rationale for MET and VEGFR inhibition in prostate cancer.

Clinical Data on HGF/MET and VEGF/VEGFR Pathway Inhibition in Prostate Cancer

Figure 19.1 shows the multiple agents currently in development targeting the HGF/MET and/or VEGF/VEGFR pathways.

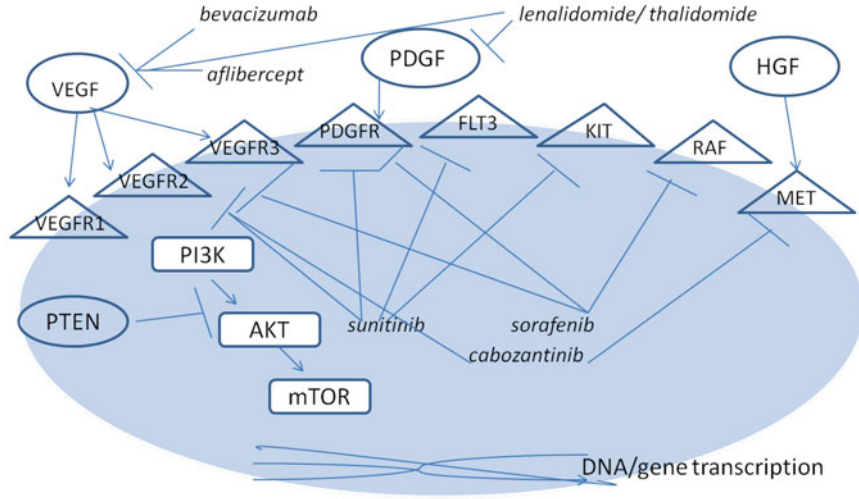

Fig. 19.1 Schematic examples of HGF/MET and/or VEGF/VEGFR inhibitors mechanism of action. *VEGFR* vascular endothelial growth factor receptor, *PDGFR* platelet-derived growth factor receptor, *HGF* hepatocyte growth factor, *FLT3* FMS-like tyrosine kinase 3, *PI3K* phosphoinositide 3-kinase, *AKT* protein kinase B, *mTOR* mammalian target of rapamycin, *PTEN* phosphatase and tensin homolog

HGF/MET

The HGF/MET axis has been targeted using both monoclonal antibodies and small molecule tyrosine kinase inhibitors (TKI). Rilotumumab (AMG102) is a fully human monoclonal antibody against HGF. A phase II trial randomized 142 men with progressive taxane-refractory CRPC to mitoxantrone (12 mg/m^2 every 3 weeks) with prednisone (5 mg twice daily) plus rilotumumab (15 mg/kg IV every 3 weeks), rilotumumab (7.5 mg/kg IV every 3 weeks) or placebo (1:1:1 randomization) [41]. Rilotumumab did not improve OS or progression-free survival (PFS) (median OS: 12.2 vs 11.1 months, HR 1.10; median PFS 3 vs 2.9 months, HR 1.02, in the combined rilotumumab vs control). Rilotumumab was well tolerated with peripheral edema (24 vs 8 %) being more common compared to control. The MET TKI tivantinib (ARQ197) is currently being studied in a randomized, blinded, placebo-controlled phase II trial in asymptomatic or minimally symptomatic metastatic CRPC. Planned enrollment is 78 patients and an estimated primary completion date of July 2014 (NCT01519414). Another small molecule MET inhibitor (AMG 208) was tested at a dose ≤400 mg daily in a phase I study and had manageable toxicities with evidence of anti-tumor activity, especially in prostate cancer [42]. Several other compounds targeting HGF/MET signaling are in early phases of clinical development, including the monoclonal antibodies onartuzumab (MetMab), TAK-701, and ficlatuzumab (SCH900105) and small molecule TKIs SGX523, PF-04217903, EMD 1214063, EMD 1204831, PF-02341066 (crizotinib), BMS-777607, SAR125844, JNJ 38877605.

VEGF/VEGFR

Four anti-angiogenesis compounds have been tested in phase III trials in CRPC; none, however, improved OS. A fifth agent is currently undergoing phase III evaluation (Table 19.1).

Bevacizumab

Based on phase II data from trials of docetaxel, bevacizumab, thalidomide, and prednisone, as well as bevacizumab combined with docetaxel/

Table 19.1 Phase III trials with HGF/MET and/or VEGF/VEGFR inhibitors in patients with CRPC

Trial identifier	Agent	Disease	Number of patients	Arms	Primary endpoint	Outcome
(CALGB 90401) NCT00110214	Bevacizumab	Metastatic CRPC (chemotherapy-naive)	1,050	Docetaxel/prednisone + bevacizumab 15 mg/kg IV every 3 weeks or placebo	OS	No difference in OS
NCT00676650	Sunitinib	Metastatic CRPC (chemotherapy-treated)	873	Sunitinib 37.5 mg po daily or placebo	OS	No difference in OS
NCT01234311	Tasquinimod	Metastatic CRPC (chemotherapy-naive)	1,200 (target)	Tasquinimod (0.25, 0.5, 1 mg po daily) or placebo	PFS	Ongoing
(VENICE) NCT00519285	Aflibercept	Metastatic CRPC (chemotherapy-naive)	1,224	Docetaxel/prednisone + aflibercept (6 mg/kg every 3 weeks) or placebo	OS	No difference in OS
(MAINSAIL) NCT00988208	Lenalidomide	Metastatic CRPC (chemotherapy-naive)	1,059	Docetaxel/prednisone + lenalidomide (25 mg po daily, days 1–14) or placebo	OS	No improvement in OS
(COMET-1) NCT01605227	Cabozantinib	Metastatic CRPC (treated with docetaxel and either abiraterone or enzalutamide)	960 (target)	Cabozantinib 60 mg po daily or prednisone 5 mg po twice daily	OS	Ongoing
(COMET-2) NCT01522443	Cabozantinib	Metastatic CRPC (treated with docetaxel and either abiraterone or enzalutamide)	246 (target)	Cabozantinib 60 mg po daily or mitoxantrone/prednisone (maximum of 10 infusions of chemotherapy or placebo)	Confirmed pain response	Ongoing

estramustine [43, 44], CALGB 90401 randomized 1,050 patients with progressive, metastatic CRPC to docetaxel (75 mg/m^2 every 3 weeks), prednisone (5 mg twice daily), and either bevacizumab (15 mg/kg IV every 3 weeks) or placebo [45]. There was no significant difference in OS (22.6 vs 21.5 months, respectively; HR 0.91; $p=0.181$). Median PFS (9.9 vs 7.5 months; $p<0.001$) and objective response rate (49.4 vs 35.5 %; $p=.0013$), as well as treatment-related toxicity (75.4 vs 56.2 %; $p\leq.001$) and treatment-related deaths (4 vs 1.2 %; $p=.005$) were higher in the bevacizumab arm. Enrollment of patients with more co-morbidities in the bevacizumab arm and treatment duration were suggested as potential reasons for the discordant PFS and OS in this trial [46, 47]. Patients discontinued bevacizumab at the time of PSA or radiographic progression before consensus guidelines were implemented discouraging discontinuation of therapy on the basis of PSA progression alone without clinical progression. The analysis of prospectively collected biomarkers in this study may identify a subset of patients who may have benefited from the combination therapy.

Sunitinib

Sunitinib showed anti-tumor activity in two phase II trials of patients with metastatic CRPC who had progressed on docetaxel [48, 49]. Unplanned post-hoc analyses of bone scans from one of these trials noted a relatively high rate of bone scan response to sunitinib, with none of patients with bone scan response experiencing concordant lowering of PSA or CT evidence of response by accepted criteria [50]. A phase III

trial randomized 873 men with metastatic CRPC progressive after docetaxel to sunitinib (37.5 mg daily) or placebo (2:1 ratio). Results from a second interim analysis reported PFS but no OS improvement (median OS 13.1 vs 12.8 months, respectively; HR 1.03; $p=0.58$) and the trial was terminated due to futility [51]. The most common treatment-related grade 3/4 adverse events were fatigue (18.8 vs 7.3 %) and anemia (6.2 vs 5.5 %). A strategy of sunitinib maintenance (50 mg daily on 4/2 week on/off cycle) after response to docetaxel was investigated in a phase II trial [52]. Three grade 4 adverse events occurred in the 23 men enrolled: hepatitis, myelosuppression, pneumonia. Most men had immediate PSA increase without evidence of disease progression. Although sunitinib was well tolerated with predictable toxicity, median PFS of 133 days was lower than the predefined threshold of 180 days. PSA level was not informative, since significant increases were noted as early as second cycle. Finally, a phase I/II trial evaluated sunitinib (2 weeks on, 1 week off) in combination with docetaxel/prednisone in chemotherapy-naive metastatic CRPC [53]. The recommended phase II dose of sunitinib was 37.5 mg daily, with standard dose chemotherapy. During the phase II portion, confirmed PSA response occurred in 56.4 % of patients. Median time to PSA progression was 9.8 months; 42.4 % of assessable men had confirmed partial response. Median PFS and median OS were 12.6 and 21.7 months, respectively. The most frequent treatment-related grade 3/4 adverse events were neutropenia (53 %; 15 % febrile) and fatigue/asthenia (16 %).

Tasquinimod

Tasquinimod is an oral quinolone-3-carboxamide derivative with anti-angiogenic properties resulting in a decrease vascular density. The mechanism of anti-tumor activity is not completely known [54]. In a double-blinded phase II trial, 201 men with metastatic CRPC were randomized to either tasquinimod 1 mg daily after a titration phase (0.25 mg daily for 2 weeks followed by 0.5 mg daily for 2 weeks) or placebo [54]. After 6 months therapy, the blind was broken and asymptomatic men on placebo and those on tasquinimod without disease progression were offered open-label treatment. Six-month progression-free proportion was higher with tasquinimod (69 vs 37 %, $p<0.001$) and median PFS was longer (7.6 vs 3.3 months, $p=0.0042$). Updated results showed the time to symptomatic progression to be longer with tasquinimod ($p=0.039$, HR = 0.42) [55]. Median time to death (34.2 vs 30.2 months) favored tasquinimod particularly in the bone-metastatic subgroup (34.2 vs 25.6 months). These results led to an ongoing phase III trial in men with asymptomatic or mildly symptomatic metastatic chemotherapy-naive CRPC. A total of 1,200 men will be randomized (2:1) to tasquinimod or placebo (NCT01234311); PFS is the primary and OS the secondary endpoint. The estimated study completion date is January 2016. Another study (CATCH) attempts to determine the safety, tolerability, and recommended dose of tasquinimod in combination with cabazitaxel/prednisone in men with chemotherapy-refractory metastatic CRPC (NCT01513733). A third study evaluates the role of maintenance tasquinimod in patients with metastatic CRPC not progressing after docetaxel (NCT01732549).

Aflibercept

Aflibercept is a fusion protein composed of the extracellular domains of VEGFR-1/2 and the constant region (Fc) of the human IgG1 antibody. Aflibercept acts as a decoy receptor, competitively binding VEGF. A phase I dose-escalation trial combined aflibercept with docetaxel in men with advanced solid tumors with preliminary evidence of anti-tumor activity in several tumor types including prostate cancer [56]. Without further assessment of this combination in a prostate cancer-specific phase II study, the VENICE phase III trial randomized 1,224 men with CRPC to docetaxel (75 mg/m^2 every 3 weeks)/prednisone (10 mg daily) and either aflibercept (6 mg/kg once every 3 weeks) or placebo [57]. With a median follow-up of 35.4 months and 873 deaths, there

was no difference in time to skeletal-related events, PFS and OS in the two arms, and there was a higher incidence of all-grade hypertension, stomatitis, appetite disorders, diarrhea, dehydration, epistaxis, dysphonia, cough, headache, and infections in the aflibercept arm.

Lenalidomide

Lenalidomide is an oral immune-modulatory compound which also inhibits VEGF signaling and angiogenesis [58]. It has a favorable safety profile compared to thalidomide, and showed activity as single agent in non-metastatic biochemically recurrent prostate cancer [59]. A double-blinded phase III trial (MAINSAIL) randomized 1,059 men with chemotherapy-naive, progressive, metastatic CRPC to docetaxel (75 mg/m^2 every 3 weeks) with prednisone (5 mg twice daily) and either lenalidomide (25 mg daily, days 1–14) or placebo [60]. In November, 2011, the data monitoring board recommended that the trial is stopped, since it was unlikely to meet its primary endpoint of OS. The median OS was 77 weeks in the lenalidomide arm; the median was not reached in the placebo arm. Treatment with lenalidomide was associated with more neutropenia, febrile neutropenia, and diarrhea. A phase II trial in men with chemotherapy-naïve metastatic CRPC combined lenalidomide and bevacizumab with docetaxel and prednisone in [61]. PSA response >50 % was reported in 85.2 % of patients (N = 54) with objective radiological response in 86.7 % of patients with measurable disease. Most common grade ≥2 adverse events included neutropenia (63 %), anemia (43 %), thrombocytopenia (13 %), hypertension (22 %), and jaw osteonecrosis (22 %). This phase II trial used enoxaparin and peg-filgrastim prophylactically to prevent thromboembolic events and neutropenia and thus maintain patients on therapy [62]. These supportive care measures allowed the combination therapy to be administered for more cycles compared to the MAINSAIL and CALGB 90401 trials potentially resulting in longer PFS, ORR, and possibly OS with an improved adverse event profile. These data suggest that supportive measures may improve the success of intensive combination regimens and are hypothesis-generating for future combination therapy trials. Ongoing trials are further assessing the role of lenalidomide in men with CRPC.

Sorafenib

Sorafenib, a multi-targeted TKI, is FDA-approved for the treatment of metastatic renal cell carcinoma and advanced hepatocellular carcinoma. Several phase II trials have evaluated sorafenib monotherapy in metastatic CRPC reporting modest activity with some discordance between PSA and radiographic response [63–66]. A phase II trial of sorafenib combined with bicalutamide in 39 men with chemotherapy-naive CRPC reported that 47 % of patients had either PSA response or stable disease ≥6 months [67]. PSA decline of ≥50 % occurred in 32 % of assessable patients, including 26 % with prior anti-androgen therapy. Median time to treatment failure was 5.5 months; grade ≥3 adverse events included fatigue, skin rash, and hand-foot syndrome. A phase II study of sorafenib and docetaxel in men with metastatic CRPC was launched but its status is unknown (NCT00589420).

Other Agents

Vandetanib is an oral agent which selectively inhibits VEGFR and epidermal growth factor receptor (EGFR). In men with metastatic CRPC, vandetanib combined with docetaxel/prednisone did not provide any benefit when compared to placebo [68]. The combination of vandetanib with bicalutamide in CRPC is currently being evaluated (NCT00757692, NCT00659438). A phase II trial reported that cediranib, a highly potent VEGFR inhibitor, was well tolerated with some anti-tumor activity in heavily pre-treated men with metastatic CRPC who had progressed after docetaxel-based therapy [69]. A randomized phase II study of docetaxel/prednisone with or without cediranib in men with chemotherapy-naive metastatic CRPC reported that the addition of cediranib to chemotherapy had increased toxicity but may have been

associated with higher rate of PSA response and clinical response [70]. Two trials are currently evaluating cediranib in the management of men with metastatic CRPC (NCT01260688, NCT00436956). A phase I study evaluated nintedanib (BIBF 1120), an oral, potent, multi-targeted TKI with anti-VEGFR activity, in combination with standard dose of docetaxel/prednisone in men with metastatic, chemotherapy-naïve CRPC, and reported that 200 mg twice daily was the nintedanib recommended dose [71]. This combination was well tolerated, with preliminary signal of efficacy and no indication of pharmacokinetic interaction between nintedanib and docetaxel. Another study investigated ramucirumab (IMC-1121B, human monoclonal antibody against VEGFR2) vs IMC-A12 (monoclonal antibody against IGF-1R) with mitoxantrone/prednisone in men with prostate cancer progressive on docetaxel [72]. Ramucirumab/mitoxantrone/prednisone was reasonably tolerated, while composite PFS and OS endpoints appeared encouraging. Pazopanib (VEGFR multi-targeted TKI), TRC105 (chimeric IgG1 k monoclonal antibody binding to human endoglin), and AMG386 (selective angiopoietin 1/2 neutralizing peptibody; recombinant peptide-FC fusion protein) are also being evaluated in clinical trials in men with prostate cancer (NCT00486642, NCT00454571, NCT01090765, NCT01553188).

MET/VEGFR

As noted above, cabozantinib is the agent targeting both of these pathways which is furthest along the development pathway. Other therapies that inhibit both MET and VEGFR signaling are in early phases of clinical development, including foretinib (GSK1363089), golvatinib (E7050), GSK1363089 (XL880), and MGCD265.

Cabozantinib

Based on phase I data, a phase II randomized discontinuation study was conducted in nine selected tumor types (study XL184-203), including CRPC. Patients received cabozantinib 100 mg daily during a 12-week lead-in stage. Patients with response by mRECIST criteria continued open-label cabozantinib, those with disease progression discontinued therapy, while those with stable disease were randomized to either placebo or cabozantinib [73]. The primary endpoint for the lead-in stage was response rate by mRECIST criteria; the primary endpoint for the randomized stage was PFS. In the CRPC cohort, a total of 171 men with progressive metastatic disease, measurable by mRECIST criteria, with $\leq$1 prior standard chemotherapy regimen and maintained on androgen deprivation, were enrolled. Randomization in this cohort was suspended after the first 122 men had been accrued due to unexpected high rate of bone scan response at the lead-in stage. Bone disease was present in 87 % of patients at baseline, while 43 % were previously treated with docetaxel. Median duration of cabozantinib therapy excluding patients randomly assigned to placebo was 4.2 months (0.5–17.2). A total of 5 % of patients had a confirmed partial response within the first 12 weeks, with 75 % having stable disease. In addition, four patients with stable disease at week 12 had a confirmed partial response after the lead-in stage. Moreover, 72 % of patients had at least one assessment showing reduction of soft tissue lesions; change in measurable disease was independent of prior treatment. Improvement in bone scan was documented in 68 % of men, including 12 % with complete resolution. These responses correlated with clinical benefit, with 67 % of assessable men reporting decrease in pain, and 56 % reporting decrease in or discontinuation of narcotics. PSA changes did not correlate with the anti-tumor effects in soft tissue and bone. All men had at least one adverse event, and most experienced more than one. The most common all-grade adverse events included a cluster of symptoms consisting of fatigue, decreased appetite, taste alterations, nausea, diarrhea, weight loss, and PPE (hand-foot syndrome) that resulted in dose reductions in 62 % of patients. These events were mostly managed with drug interruption and/or dose reduction. Most common grade 3 adverse events were fatigue (16 %), hypertension (12 %), PPE (8 %), dehydration (8 %), pulmonary

embolism (7 %), decreased appetite (6 %) and nausea (5 %). The authors concluded that cabozantinib had substantial anti-tumor activity in men with advanced CRPC with manageable toxicity.

A phase II single-institution trial assessed the efficacy and tolerability of cabozantinib at lower starting doses (60, 40, 20 mg) with daily administration [74]. The primary endpoint was bone scan response in 6 weeks, defined as ≥30 % decrease in bone scan lesion area; the secondary endpoint was change in circulating tumor cells (CTC). Partial responses were seen in 15/24 evaluable patients enrolled at 40 mg dose, with one complete response and eight with stable disease. In ten men enrolled at 20 mg dose, there was one partial response, five men had stable disease, and four had disease progression. No patient required dose reduction or interruption at 6 or 12 weeks; three men at 40 mg daily discontinued due to adverse events by 12 weeks. At 40 mg dose, median treatment duration was 27 weeks; 58 % of men with ≥5 CTCs/7.5 mL at baseline converted to <5 CTCs/7.5 ml.

Additional studies are ongoing attempting to explore the underlying mechanisms of cabozantinib activity. One study of the combination of cabozantinib, docetaxel, and prednisone is examining changes in soluble MET, angiogenic factors, bone-specific alkaline phosphatase, and tumor specific MET signaling [75]. Preliminary reports suggest changes in soluble markers of MET, bone turnover, and angiogenesis correlated with cabozantinib activity. Analyses of bone marrow samples showed high MET activation in pre-treatment metastases and reported cabozantinib-mediated inhibition at 6 weeks. The authors concluded that MET may contribute to "driver" signaling networks in metastatic CRPC and that the study of stroma cell biomarkers should be further pursued. A phase II trial designed to characterize its effects on bone metabolism and tumor activity in prostate cancer bone lesions, utilizing perfusion/diffusion-weighted MRI to generate parametric response maps as well as targeted bone lesion biopsies is currently ongoing [76]. The primary endpoint of this study is the proportion of men who remain progression-free at 12 weeks; secondary endpoints include safety, PFS, response proportion and duration, PSA response, PSA time-to-progression. Four additional studies aim to explore the changes in bone and visceral metastases, and tumor imaging during treatment with cabozantinib in men with metastatic CRPC (NCT01812668, NCT01703065, NCT01599793, NCT01834651).

A phase I trial aims to evaluate the safety and recommended phase II dose of cabozantinib combined with a fixed dose of docetaxel/prednisone in men with metastatic CRPC [77]. Three escalating doses (20, 40, 60 mg) daily of cabozantinib are being tested. An expansion cohort will be enrolled at the maximum tolerated dose. Secondary objectives include assessments of pharmacokinetics of each agent, evaluation of anti-tumor activity of the combination therapy, and assessment of changes in molecular biomarkers for receptor tyrosine kinase and angiogenesis pathways, as well as biomarkers for bone metabolism. Another phase I study aims to assess the safety of the combination of cabozantinib with the anti-androgen abiraterone in men with CRPC (NCT01574937).

Two phase III, randomized, double-blinded, controlled trials of cabozantinib (COMET-1, COMET-2) at starting dose of 60 mg daily are currently accruing patients with metastatic CRPC and disease progression after prior treatment with docetaxel and either abiraterone or enzalutamide. COMET-1 (NCT01605227) compares cabozantinib with prednisone, with the primary endpoint being OS. COMET-2 (NCT01522443) compares cabozantinib with mitoxantrone/prednisone in men with symptomatic disease, with the primary endpoint being confirmed, durable pain response from week 6 to week 12.

Conclusion

Despite the biologic role and the preclinical data supporting HGF/MET and VEGF/VEGFR inhibition in CRPC, strategies of targeting either pathway alone have not resulted in significantly improved outcomes. However, combined targeting of MET/VEGFR2 with cabozantinib showed promising activity in a phase II randomized

discontinuation trial, and this agent is currently being tested in two phase III trials. Understanding of underlying disease biology and tumor-stroma interactions, development of biomarkers predictive of response, and elucidation of resistance mechanisms will result in better patient selection and undoubtedly improve the success rate of targeted therapies in this disease.

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Epigenetics in Castration Resistant Prostate Cancer

20

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Introduction

Castration resistant prostate cancer (CRPC) is a heterogeneous disease state. Until recently, treatment options for CRPC were limited to taxane chemotherapy. However, exciting advancements have resulted in the addition of five new agents for the clinical management of CRPC. While these new therapies have expanded treatment options for patients, sustainable suppression of CRPC growth still remains a primary challenge in the clinic, and novel therapeutic options are still required. Epigenetic mechanisms, including DNA methylation, histone modifications, and microRNA (miRs), have been demonstrated to play an important role in CRPC. Rapid development of inhibitors towards these epigenetic mechanisms has given rise to their exciting potential to treat patients with CRPC. This chapter will discuss these epigenetic mechanisms and their potential as targets for novel therapeutic strategies for CRPC.

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Role of Histone Acetylation in CRPC

Histone Deacetylases (HDACs)

Histone acetylation and deacetylation govern chromatin structure and regulate gene expression (Fig. 20.1). The histone writers, histone acetylases (HATs), add acetyl groups to lysine residues within histone tails, resulting in less compact structure that allows transcription factors and coactivators to drive gene expression. Further, the erasers, histone deacetylases (HDACs), remove acetyl groups from histones tails, leading to compressed chromatin structure that represses gene transcription. HDACs exist within four classes classified according to their homology to yeast proteins, their cellular location, and enzymatic activity: Class I (HDAC1, 2, 3, and 8), class II (HDAC 4, 5, 6, 7, 9, and 10), class III (SIRT1, 2, 3, 4, 5, 6, and 7), and class IV (HDAC11). Class I, II, and IV HDACs are zinc-dependent deacetylases. Class III HDACs are NAD^+-dependent and are not inhibited by conventional HDAC inhibitors. In addition, HATs and HDACs also manipulate the functions of non-histone proteins, such as p53, Heat Shock Protein 90 (Hsp90), α-tubulin, and the androgen receptor (AR). Imbalance between HATs and HDACs results in aberrant gene expression, leading to cancer development. HDACs overexpression, for instance, has been shown in colon, breast, prostate, and hematological cancers to drive disease progression [1, 2].

F. Saad and M.A. Eisenberger (eds.), *Management of Castration Resistant Prostate Cancer*, Current Clinical Urology, DOI 10.1007/978-1-4939-1176-9_20,

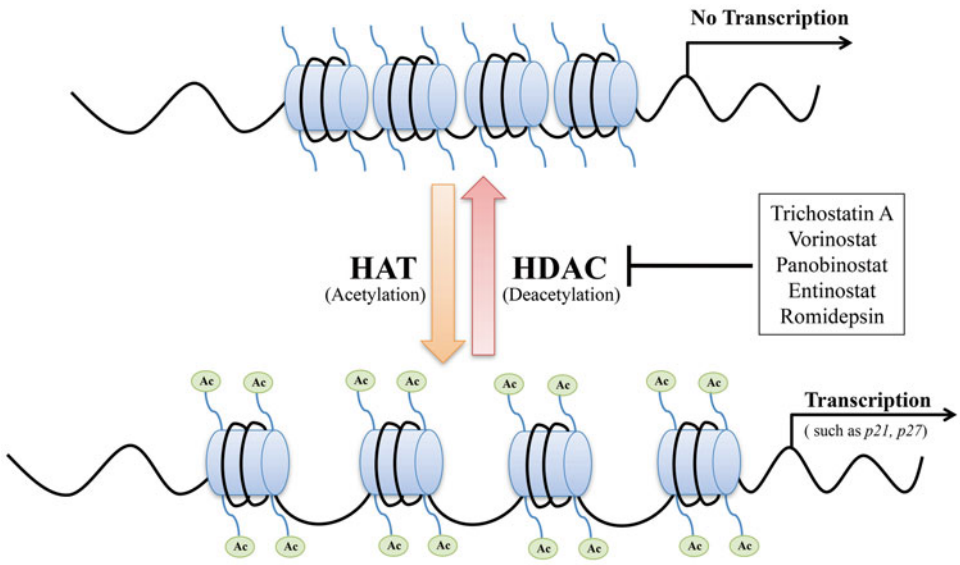

Fig. 20.1 *The mechanism of histone acetylation and deacetylation.* The modifications of histones are able to regulate gene transcription. Histone acetylase (HAT) adds acetyl groups onto the lysine tail of histones, leading to the open structure that activates the gene transcription, such as *p21* and *p27*. Histone deacetylase (HDAC), however, removes acetyl groups resulting in compact structures to repress gene expression. HDAC inhibitors, vorinostat, TSA, panobinostat, entinostat, and romidepsin, inhibit HDAC activity, allowing gene expression to induce apoptosis. *Ac* acetyl group, *HAT* histone acetylase, *HDAC* histone deacetylase

As stated, deacetylation of histones gives rise to compact chromatin structure to prevent gene expression, including tumor suppressor genes, p21 and p53, leading to uncontrolled cell growth [1]. HDAC1 overexpression, for example, has been demonstrated in castration-resistant disease. HDAC1 up-regulation not only facilitates cell proliferation, but also induces the loss of luminal epithelial cytokeratin 18 (CK18), with concurrent up-regulation of cytokeratin 5 (CK5), a marker of progenitor basal cells [3] (Table 20.1). Additionally, a recent study concluded that the knockdown of HDAC1 or HDAC3 resulted in suppression of AR-regulated genes (ARG), such as KLK2, PSA, and NKX3.1, but not TMPRSS2, suggesting that HDAC1 and HDAC3 are required for ARG expression [4]. Another class I HDAC, HDAC2, was investigated in a cohort of 192 prostate cancer patients that high expression of HDAC2 was correlated with shorter relapse-free survival [5]. Welsbie et al. showed that HDAC2 does not regulate ARG expression, suggesting that the role of HDAC2 in prostate cancer development may not involve AR transcriptional activity. Moreover, the activity of HDAC 1 and 2 is positively correlated with Gleason score, indicating that HDAC1 and HDAC2 could be potential prognostic markers in prostate cancer [5]. HDAC4 is an androgen-regulated class II HDAC. Its nuclear translocation is induced by ligands in benign prostatic hyperplasia and primary prostate cancer. In CRPC, however, it is predominately located in the nuclear compartment, to drive aggressive disease progression by inhibiting differentiation [6]. HDAC6 is the major HDAC to govern the activity of molecular chaperone Heat Shock Protein 90 (Hsp90). A critical role of Hsp90 in PCa cells is to mediate AR nuclear translocation and transcriptional activity. Ai et al. demonstrated that HDAC6 knockdown prevents AR nuclear localization through acetylating Hsp90, suggesting that HDAC6 plays an important role in AR hypersensitivity in CRPC [7]. Sirtuin 1 (SIRT1), a class III HDAC, has been exhibited in association with cell proliferation and chemo resistance in PC3

Table 20.1 The list of HDACs in prostate cancer

Class	Members	Localization	Status in prostate cancer
Class I	HDAC1	Nucleus	Overexpression
	HDAC2	Nucleus	Overexpression
	HDAC3	Nucleus	Overexpression
	HDAC8	Nucleus	Unclear
Class II	HDAC4	Nucleus/ cytoplasm	Overexpression
	HDAC5	Nucleus/ cytoplasm	Unclear
	HDAC6	Cytoplasm	Induce AR hypersensitivity
	HDAC7	Nucleus/ cytoplasm	Unclear
	HDAC9	Nucleus/ cytoplasm	Unclear
	HDAC10	Cytoplasm	Unclear
Class III	SIRT1	Nucleus	Suppress PC3 and DU145 cell growth
	SIRT2	Cytoplasm	Unclear
	SIRT3	Nucleus/ mitochondria	Unclear
	SIRT4	Mitochondria	Unclear
	SIRT5	Mitochondria	Unclear
	SIRT6	Nucleus	Unclear
	SIRT7	Nucleus	Unclear
Class IV	HDAC11	Nucleus/ cytoplasm	Unclear

and DU145. Kojima et al. demonstrated that suppressing SIRT1 in PC3 and DU145 is able to repress cell proliferation and enhance the sensitivity to camptothecin and cisplatin [8]. Other HDACs and their involvement in CRPC remain unclear. Ongoing studies within prostate cancer and other malignancies will allow us to eventually fully elucidate HDAC involvement in CPRC, and how this knowledge can be applied to novel therapeutic strategies involving HDAC inhibition.

Histone Deacetylase Inhibitors (HDACi) in CRPC

Several agents have been developed to target HDACs. These inhibitors exhibit pleiotropic effects including cell cycle arrest, DNA damage, autophagy, and apoptosis [9–11]. To date, there are at least 18 HDAC inhibitors (HDACi), divided into seven classes, being investigated in prostate cell lines or animal models [1]. In particular, vorinostat, entinostat, and panobinostat are being investigated in clinical trials.

Vorinostat, also named as suberoylanilide hydroxamic acid (SAHA), is a hydroxamate compound inhibiting class -I, -II, and -IV HDAC and has been approved to treat cutaneous T cell lymphoma by FDA in 2006 [1]. In prostate cancer therapy, it is able to induce cell death in vitro in LNCaP, LAPC4, CWR22, DU145 [12], and reduce CWR22 tumor burden in vivo [13]. Molecular analysis shows that vorinostat represses AR, PSA, and KLK2 transcription, but does not affect AR degradation [14]. Moreover, numerous studies displayed that vorinostat arrests cell cycle in G2/M, repressing EGFR expression, and has been reported to have synergistic effect with bicalutamide in DU145 [14].

Trichostatin A (*TSA*) is an antifungal antibiotic, specifically blocking mammalian class I and II HDACs [1]. One study showed that TSA induces cell death in LNCaP and CWR22R, but not in PC3 and DU145 [15]. Suenaga et al. suggested that TSA is capable of suppressing telomerase reverse transcriptase (hTERT) mRNA, which is responsible for maintaining telomere integrity, to restrain LNCaP and PC3 cells proliferation [16].

Panobinostat is a derivative of hydroxamic acid, the same class as SAHA. It has great activity against class -I, -II, and -IV HDAC at nanomolar ranges [1]. Some studies have indicated that panobinostat induces cell apoptosis in LNCaP [4], MYC-CaP/AS [17], MYC-CaP/CR [17], as well as PC3-AR expressing cells [18] and decreases tumor size of MYC-CaP [17], CWR22RV1 [4], PC3-AR [18] in vivo models. Several research teams have revealed that panobinostat blocks AR transcriptional activity, induces Caspase-3 activations, and attenuates ATM–Akt–ERK DNA damage pathway to cause cell death [4, 17, 18].

Romidepsin, also named as FK228, belongs to the class of bicyclic and capable of releasing a zinc-binding thiol in a cell to repress HDAC activity [1]. Sasakawa et al. have indicated that romidepsin inhibits PC3 and DU145 cell growth as well as tumor burden by inducing p21 and downregulating c-Myc expression [19]. Besides,

Table 20.2 The list of the main HDAC inhibitors in vitro studies

Name of HDAC inhibitors	Combined treatment	Cell lines/animal models	Conclusions	Reference
Vorinostat	RMT5625; HA14-1	LNCaP; DU145; LAPC4; PC3	Induce cell death in LNCaP and DU145, but not in PC3	[12]
		CWR22 xenograft	Reduce tumor burden	[4]
	Bicalutamide	LNCaP; PC3	The combination increases cell death in LNCaP, but not in PC3	[14]
TSA	Doxorubicin	LNCaP; CWR22; PC3; DU145	The combination enhance cell death in LNCaP and CWR22	[15]
		LNCaP; PC3	Increase p53 acetylation	[23]
		LNCaP; PC3	Downregulate telomerase reverse transcriptase	[16]
Panobinostat	Everolimus	MYC-CaP	Concurrent treatment increase cell apoptosis and inhibit tumor growth and AR transcriptional activity that this study also inhibited HIF-1 alpha transcription activity	[17]
	BEZ235	PC3; PC3AR	Suppress tumor growth in both PC3 and PC3-AR xenografts by inhibiting AKT–mTOR–ERK pathway increases double strand breaks of DNA increases inhibition of ATM	[18]
		CWR22Rv1	Inhibit tumor growth	[4]
Romidepsin		PC3; DU145	Repress tumor burden in PC3 and DU145 by increasing p21 expression and decreasing c-Myc	[19]
		CWR22Rv1	Prolong survival rate	[20]
Entinostat		LNCaP; PC3; DU145; TRAMP	Suppress tumor growth of LNCaP, DU145 and PC3. Prevent tumor progression in TRAMP	[21]
	Radiation	DU145	Enhance the radiosensitivity in vivo	[22]

the other group displayed that romidepsin can repress tumor volume in the 22Rv1 model and prolong survival rate [20].

Entinostat is a benzamide-based HDACi and targeting class I HDAC exclusively. It has been approved by FDA for ER-positive breast cancer treatment and investigated in LNCaP, PC3, as well as DU145 cells showing anti-tumor effect. [21]. Moreover, a study concluded that entinostat acts as a radiosensitizer to enhance radiation mediated DNA double strand breaks (DSB), as indicated by increased levels of gamma-H2AX [22]. Importantly, Qian et al. demonstrated that entinostat significantly prevents the progression of prostate tumorigenesis in TRAMP model [21].

Combination Strategies with HDAC Inhibitors in CRPC

The efficacy of HDAC inhibitors as single agents has been demonstrated in various prostate cancer cell lines and in vivo models. However, the development of CRPC results from multiple signaling pathways [24] (Table 20.2). For this reason, combining HDACi with other agents has been studied in preclinical PCa animal models and clinical trials in patients with PCa. Marrocco et al. combined vorinostat with the AR antagonist, bicalutamide, showing synergistic cell killing effect in the LNCaP cell line [14]. Also, Roklhin et al. displayed the synergistic effect of TSA and doxorubicin in LNCaP and CWR22R cells [15]. Moreover, due to the fact that panobinostat results in PI3K–AKT–mTOR pathway repression, Ellis et al. adopted Myc-CaP and PC3 models to demonstrate the greater therapeutic efficacy in vitro and in vivo by combining panobinostat with the specific mTORC1 inhibitor everolimus and the PI3K–mTOR dual inhibitor BEZ235 [17, 18].

The promising results from these in vitro and preclinical in vivo studies involving HDAC inhibitors in various PCa models are encouraging. These results have led to numerous clinical trials, investigating the potential of HDAC

Table 20.3 The list of HDAC inhibitor in clinical trials

Name of HDAC inhibitors	Combined therapy	Phase	Status	Dose/schedule	Conclusion	Reference
Vorinostat (SAHA)		II	Completed	400 mg/day, orally	2/27 patients stable disease; significant toxicities and limiting efficacy	[27]
		I	Completed	Dose escalation study	Unpublished	NCT00005634
		I	Completed	Dose escalation study	Unpublished	NCT00045006
	Docetaxel	I	Terminated	SAHA: 100-500 mg/day, orally; Docetaxel: 50-75 mg/m^2	Excessive toxicity	[28]
	Temsirolimus	I	Ongoing	400 mg qd	N/A	NCT01174199
	Doxorubicin	I	Completed	SAHA: 400-1000 mg/day; Doxorubicin: 20 mg/m^2	1/2 patients partial response	[29]
Panobinostat (LBH589)		II	Completed		Unpublished	NCT00667862
	Radiotherapy	I	Completed		Unpublished	NCT00670553
	Docetaxel	I	Completed	LBH589: 15 mg/MWF, orally; Docetaxel: 75 mg/m^2	2/7 patients partial response with a 50 % decline in PSA; 4/7 patients stable disease	[26]
	Bicalutamide	I/II	Ongoing	LBH589: 60 or 120 mg/week, orally; Bicalutamide: 50 mg/day, orally	N/A	NCT00878436
Entinostat (MS-275	13-cis retinoic acid (CRA)	I	Completed	MS-275: 4 mg/m2 weekly; CRA: 1 mg/kg/day, orally	Well tolerated	[30]

inhibitors as single agent or in combination with radiotherapy, bicalutamide, docetaxel, doxorubicin, and temsirolimus [25] (Table 20.3). An example of a promising clinical trial involved the treatment of patients with docetaxel and panobinostat demonstrating PSA reduction [26]. Although most trials are still ongoing and vorinostat single treatment in CRPC has been reported high toxicity, low dose of HDAC inhibitors or combining with other drugs may still provide an avenue to treat CRPC patients.

Methylation in CRPC

Like acetylation and deacetylation of histones, methylation of DNA and histones are reversible alterations that lead to stable inheritance of cellular phenotypes without any changes in the DNA sequence (Fig. 20.2). During DNA methylation process, the methyl group ($-CH_3$) is supplied by S-adenosylmethionine (SAM) and transferred to the 5′-carbon of a cytosine base of a CpG dinucleotide. Regions characterized by high frequency of CpG sites are defined as CpG islands and their methylation acts as a stable tag on gene promoters, leading to the formation of large-scale heterochromatic structures that silence the associated genes.

Histone methylation occurs on arginine and lysine residues of histone tails. The methylation is catalyzed by histone methyltransferase enzymes and a single lysine residue can be methylated up to three times leading to either gene repression and activation. Both DNA and histone methylation can drive to genetic instability and alteration in normal gene transcription and have been implicated in a variety of human cancers, including PCa [31]. For those reasons, these epigenetic modifications can potentially be used for the molecular classification, detection, risk assessment, and treatment in prostate cancer.

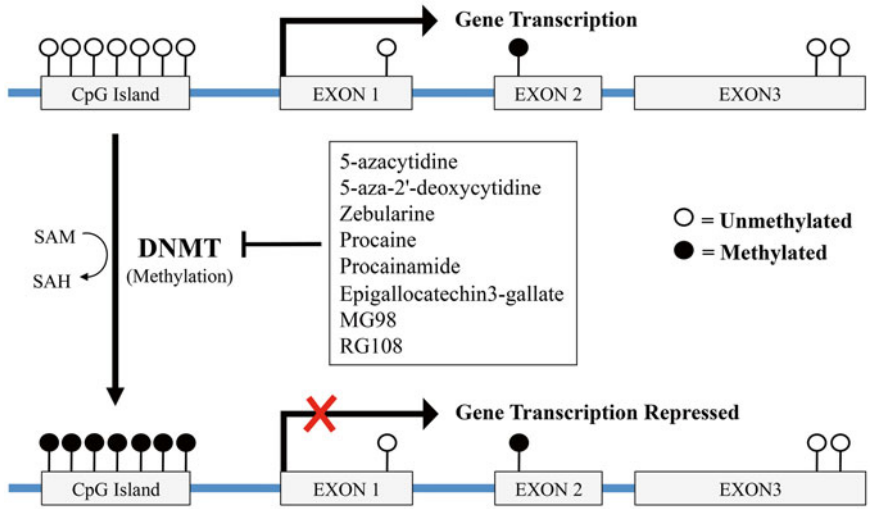

Fig. 20.2 *Schematic representation of DNA methylation and its regulatory effect on gene transcription.* During DNA methylation, DNA methyltransferases (DNMT) catalyze the addition of a methyl group ($-CH_3$) to a cytosine base within a CpG island. Hypermethylation of CpG islands located in the promoter region of a gene lead to its transcriptional silencing. DNMT inhibitors, restoring the normal methylation pattern, induce the transcription of specific genes involved in apoptosis, cytostasis, and differentiation. *DNMT* DNA methyltransferase, *SAM* S-adenosyl methionine, *SAH* S-adenosyl homocysteine

In the following sections, the principal epigenetic molecules involved in PCa progression and their relative inhibitors will be presented.

Role of DNA Methyltransferases (DNMTs) in CRPC

The DNA methyltransferases (DNMT) are a family of enzymes responsible for the establishment and differentiation of DNA methylation patterns during development. The components of this family are: DNMT1, DNMT3a, and DNMT3b. DNMT1 is considered to be the key maintenance enzyme, acting on hemi-methylated DNA substrates generated during DNA synthesis, to maintain CpG methylation patterns through genome replication and mitosis. In contrast, DNMT3a and DNMT3b perform de novo methylation. In human cancer cells, DNMT1 is responsible for both de novo and maintenance methylation of tumor suppressor genes leading to their silencing. Progressive increase in generalized DNMT enzymatic activity has been shown during malignant transformation. Gravina et al. showed that human PCa cells continuously treated with bicalutamide (BCLT) or cultured in androgen-depleted medium progressively acquire higher DNA methyltransferase (DNMT) activity and expression, mainly DNMT3a and DNMT3b, proportionally to their androgen independence [32]. These observations have also been correlated in patients, where DNMT3a and DNMT3b expression was upregulated by neoadjuvant treatment with BCLT [32]. Those results demonstrated that BCLT treatment can mediate changes in DNA methylation status thus suggesting a possible involvement in preventing the action of antiandrogen therapy. Furthermore, increased DNMT expression and activity also correlates with the up-regulation of truncated AR isoforms, which favor the development of the hormone-resistant phenotype [33].

DNA Methyltransferases (DNMTs) Inhibitors

DNMTs inhibitors represent a promising class of epigenetic modulators, showing efficient antitumorigenic activity in vitro and in vivo against

Table 20.4 List of the main DNMT inhibitors tested and their mechanism of action (adapted from Gravina GL et al. [42])

DNMT inhibitor	Mechanism of action
Nucleoside	
5-Azacytidine	Inhibit mRNA translation and when incorporated into DNA inhibits methylation by trapping DNMTs
5-Aza-2′-deoxycytidine/ decitabine	When incorporated into DNA inhibits methylation by trapping DNMTs
Zebularine	When incorporated into DNA inhibits methylation by trapping DNMTs
Non-nucleoside	
Procaine	Binds to CpG-rich sequences and block the binding of DNA methyltransferases to DNA
Procainamide	Reduces DNMT1's affinity for both DNA and S-adenosyl-methionine
Epigallocatechin3-gallate (EGCG3)	
MG98	This antisense oligonucleotide targets the 3 UTR of DNMT1
RG108	Binds to the catalytic site of DNMTs

several hematologic and solid tumors. DNMTs inhibitors are subdivided into nucleoside and non-nucleoside inhibitors (Table 20.4). Nucleoside inhibitors become incorporated into the DNA during replication and lead to the sequester of DNMTs. Non-nucleoside inhibitors do not require incorporation into DNA and are thought to be safer due to their lower mutagenic potential. Belonging to the nucleoside inhibitors are the 5-azacytidine (Vidaza) and the 5-aza-2′-deoxycytidine (or decitabine, Dacogen). 5-aza-2′-deoxycytidine is one of the first DNMT inhibitors identified. This agent forms irreversible covalent bonds with DNMT1 after its incorporation into DNA, thereby inducing degradation of DNMT1. Although different studies support the benefit of 5-aza or 5-aza-2′-deoxycytidine treatment in androgen-independent PCa cell lines [34], in CRPC patients, 5-aza-2′-deoxycytidine has been evaluated in phase II trials, but its antitumor effects resulted to be modest. Overall, 5-aza-2′-deoxycytidine led to stable disease in 17 % of patients, and median time to progression was only 10 weeks [35]. The same poor efficacy in chemonaive CRPC patients has been observed for 5-azacytidine (median progression-free survival of 12.4 weeks) [36]. Zebularine, another cytidine analog, has shown minimal acute toxic effects and higher chemical stability when compared to 5-aza-2′-deoxycytidine. Zebularine has been evaluated in preclinical studies for myeloid malignancies and selected carcinomas [37–40], but it has not yet been tested for the treatment of PCa. Non-nucleoside DNMT inhibitors like procaine and procainamide reduce DNMT affinity for both DNA and S-adenosyl-methionine causing a decrease in DNA methylation. To date, non-nucleoside analogs have been much less efficacious in inhibiting DNMT and have not been evaluated in clinical trials. However, epigallocatechin3-gallate (EGCG3), the major phenol in green tea, has been found to be a potent inhibitor of S-adenosylmethionine-dependent methyltransferase (such as DNMTs). The mechanism of its cancer-preventive action in prostate cancer is still unclear. However, a placebo-controlled, double-blind phase II trial has been conducted evaluating its ability to prevent progression towards invasive carcinoma in patients with high grade PIN lesion but no data are yet available [41]. Finally, MG98 and RG108, antisense oligodeoxynucleotides that target the 3′ UTR of DNMT1 and a small molecule able to bind to its catalytic site respectively, have demonstrated good in vitro anticancer effects with, however, a limited clinical success. Despite the promising anticancer activity in hematological malignancies, early clinical trials involving patients with solid tumors including PCa demonstrated that DNMT inhibitors have low anticancer activity and significant toxicity as monotherapy. However, recent studies suggest that low concentrations of DNMT inhibitors such as 5-Aza and decitabine may act synergistically when combined with chemotherapy and contribute to overcoming intrinsic or acquired chemoresistance in several cancer types [42].

Role of Histone Methyltransferases (HMTs) in CRPC

Histones are the main structural components of chromosomes. These proteins assemble to form octamer and act as spools around which DNA is wrapped to form a nucleosome. Histones undergo

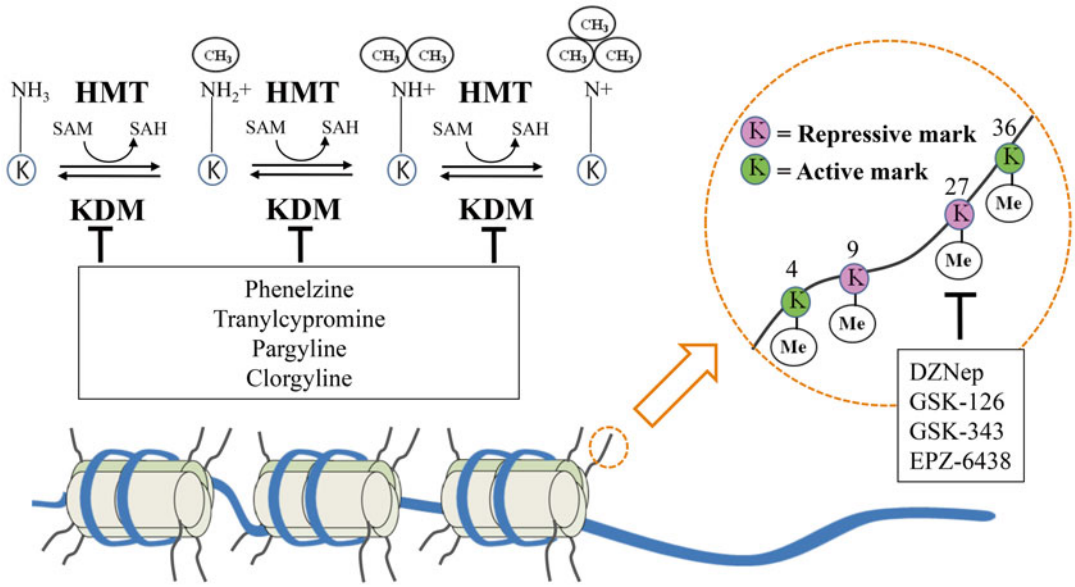

Fig. 20.3 *Histone methylation: mechanism and functions of the principal methylation sites.* Histones are the structural unit around which DNA is wrapped and packaged to form nucleosomes. Histone methylases (HMT) and demethylases (KDM) affects chromatin remodeling and function depending on the specific amino acid being modified and the extent of methylation. Active methylation marks lead to gene transcription while repressive marks induce gene silencing. Both EZH2 inhibitors (DZNep, GSK-126, GSK-343, and EPZ-6438) acting on the repressive mark on lysine 27 of histone 3, and KDMs inhibitors share promising therapeutic efficacy on the treatment of prostate cancer. *Me* Methyl group, *HMT* Histone methyltransferase, *KDM* Histone lysine demethylase, *SAM* S-adenosyl methionine, *SAH* S-adenosyl homocysteine, *EZH2* Enhancer of zeste homolog 2

a sophisticated pattern of posttranslational modifications, the histone code, that alter their interaction with DNA and nuclear proteins and are needed to regulate gene transcription. Histone methylation can affect chromatin remodeling and function depending on the specific amino acid being modified and the extent of methylation and can lead to both activation and repression (Fig. 20.3) [31]. The two histone methylation marks, therefore, associated with active transcription are the trimethylation of histone H3 at lysine 4 (H3K4Me3) and the trimethylation of histone H3 at lysine 36 (H3K36Me3). Among the repressive marks are the trimethylation of histone H3 at lysine 27, 9, and 20 (H3K27Me3, H3K9Me2/3, and H3K20Me3). Increasing evidence suggests that alterations in the histone code may play an important role during prostate tumorigenesis and the expression levels of histone methyltransferases are often altered in multiple tumor types. In 2009, two independent groups have shown that, in CRPC models, enhanced levels of the active histone marks H3K4me1/2/3 are selectively enriched at the enhancer regions of some AR-regulated cell cycle genes thus promoting CRPC cell growth and survival [43, 44]. Further, an increase in the repressive histone marks H3K27me3 has also been shown during prostate cancer progression, correlating with a poor clinical outcome. H3K27me3 increase is attributed to the overexpression of the Enhancer of Zeste Homolog 2 (EZH2) and leads to the silencing of a wide number of genes (such as tumor suppressor genes, GAS2, PIK3CG, and ADRB2), whose repression can induce cancer cell growth and invasion. EZH2 is a histone methyltransferase belonging to the polycomb repressive complex 2 (PRC2) and its overexpression has been seen in metastatic prostate cancer [45] following microRNA-101 deletion, a negative regulator [46]. Despite several studies have focused on PCR2-mediated

repression as EZH2 oncogenic function, in 2012, Xu et al. have demonstrated that EZH2 overexpression in a model of androgen-independent PCa (LNCaP-abl) correlates with a switch in EZH2 activity from gene repressor to gene activator. EZH2 was found to be highly phosphorylated on a specific serine residue (S21) and, depending on its phosphorylation, able to interact and act as an AR transcriptional co-activator. The importance of phosphorylation at S21 has also been confirmed for the androgen-independent growth of the androgen-sensitive cell line, LNCaP [47]. Moreover, H3K27me3 levels are significantly decreased in CRPC, further supporting the idea of an EZH2 oncogenic activity independently of its Polycomb repressive function [47]. A deregulated expression of EZH2 has also been described in other solid tumors such as: bladder, gastric, lung, and hepatocellular carcinoma. Interestingly, recent work showed that EZH2 can function as an upstream activator of another HMTase, MMSET/NSD2. NSD2 mediates H3K36me2, a histone mark associated with active transcription, and its expression and function has been reported to be tightly related to EZH2 in most human cancers [48]. In CRPC, NSD2 is overexpressed and acts as a strong co-activator of NF-κB, promoting cell proliferation and survival and tumor growth [49].

Histone Methyltransferases (HMTs) Inhibitors

Following those findings, small molecules targeting the histone methylation enzymes could have a significant impact on cancer treatment. Over the past years, several HMTs inhibitors have been developed and tested. Specifically, 3-Deazaneplanocin A (DZNep) is a cell-permeable compound, originally synthesized as S-adenosylhomocystein (AdoHcy) hydrolase inhibitor to indirect suppress SAM-dependent methylation, and has also been shown to deplete the levels of PRC2 components (EZH2, EED, and SUZ12). In cells of human acute myeloid leukemia (AML), DZNep treatment induced an increase of the cell cycle regulators p21, p27, and FBXO32, leading to cell cycle arrest and apoptosis. Moreover, a synergistic effect between DNZep and the HDAC inhibitor panobinostat as been demonstrated in human AML cells [50]. Among the other EZH2 inhibitors, GSK-126 and GSK-343 have been demonstrated to efficiently inhibit the growth of, respectively, diffuse large B-cell lymphoma (DLBCL) and epithelial ovarian cancer (EOC) cells. Since in hematological malignancies activating or inactivating mutations in EZH2 SET domain are often found, both inhibitors are effective against either wild type (WT) or mutant forms of EZH2. Another small molecule inhibitor (EPZ-6438) has been formulated for the treatment of non-Hodgkin lymphoma patients with oncogenic point mutation in EZH2. Based on the durable tumor regression seen in preclinical studies, this compound has been formulated to work as single-agent treatment and, in June 2013, a Phase 1/2 clinical trial of EPZ-6438 in patients with advanced solid tumors or with relapsed or refractory B-cell lymphoma has been initiated. In view of the promising results obtained in hematological disease, EZH2 inhibitors are now being tested also in prostate cancer.

Role of Histone Lysine Demethylases (KDMs) in CRPC

Histone lysine demethylases (KDMs) are enzymes able to remove both repressive and activating histone marks (Table 20.5). KDMs are grouped in seven major classes, each one targeting a specific histone methylation site. Belonging to the first class, KDM1, are two isoforms of flavin-dependent demethylases (KDM1A and B, also known as LSD1 and LSD2) responsible for the demethylation of H3K4me1/2. Since H3K4me2 is an active histone mark, KDM1 favors gene silencing. The second class of KDMs includes a cluster of six (KDM 2-7) of Fe^{2+}/oxoglutarate-dependent enzymes, containing a characteristic Jumonji C (JmjC) domain. Most of the clusters include at least two members and are characterized by one or more targets.

Table 20.5 KDMs classes and functions

KDMs class	Target histone mark	Transcriptional modulation
KDM1	H3K4me1/2	Silencing
	H3K9me2/3	Activation
KDM2	H3K36me2	Silencing
KDM3	H3K9me2	Activation
KDM4	H3K36me2/3	Silencing
	H3K9me2/3	Activation
KDM5	H3K4me2	Silencing
KDM6	H3K27me3	Activation
KDM7	H3K9me1/2 and H3K27me1/2	Activation

Table 20.6 KDMs known to be involved in PCa. (Adapted from Crea F et al. [59])

KDMs class	Expression in PCa	Role in PCa
KDM1A	Overexpressed	AR co-activator/repressor
KDM2A	Downregulated	Putative tumor suppressor
KDM3A	Overexpressed	AR co-activator
KDM4A	Overexpressed	
KDM4B	Overexpressed	
KDM4C	Overexpressed	AR co-activator
KDM5B	Overexpressed	AR co-activator
KDM5C	Overexpressed	TGFB signaling suppressor
KDM6B	Overexpressed	Putative oncogene
PHF8	Overexpressed	Mediates cell invasion

Overexpression or mutation of KDMs has been linked to mis-erased histone methyl modifications and may play oncogenic or tumor-suppressive roles in several neoplasms. Histone demethylases such as KDM5A, 5C, and 6A are involved in several malignancies (acute myeloid leukemia, esophageal, renal, and squamous cell carcinomas) and KDM1A is thought to play a role in several neoplasms including PCa [51]. In PCa, KDM1A can function as AR co-activator and its oncogenic activity is partially due to its ability to trigger Myc-dependent transcription [51], and inhibit p53 pro-apoptotic function [52]. In the presence of high androgen concentrations, KDM1A can be recruited by the AR to mediate gene silencing thus acting as co-repressor [53]. In primary PCa, high KDM1A expression predicts higher risk of tumor relapse after prostatectomy [54]. Other KDMs observed to be overexpressed in CRPC are: KDM4C, found to be required for esophageal squamous carcinomas cell proliferation [55], PHF8 implicated in PCa metastatic disease [56] and KDM5B, an AR-co-activator [57]. Conversely, KDM2A is downregulated in PCa, indicating that it could play a tumor-suppressive function through its role in maintaining genome integrity (Table 20.6) [58]. Moreover, it has been shown that a correlation between KDMs expression and different PCa progression stages indicating that KDMs could have a role as novel biomarkers for prediction of tumor-initiation, progression-free survival, and androgen-independent state [59].

Histone Lysine Demethylases (KDMs) Inhibitors

KDMs can be targeted by selective small molecule inhibitors, which are already being tested in biochemical and preclinical models. Since KDM1A catalytic domain shares homology with neural Mono Amino Oxidase (MAO), pharmacological inhibitors developed as antidepressive agents have been employed to target cancer cells. Indeed, phenelzine, tranylcypromine, and pargyline have been reported to act as KDM1A inhibitor. Pargyline has been first described as a KDM1A inhibitor in PCa cells, but further studies failed to confirm this observation. Tranylcypromine and its analogues (NCL-1 and NCL-2) have proved to more effectively inhibit H3K4me2 along with a safe toxicity profile. For this reason, tranylcypromine analogues may be particularly effective to prevent PCa recurrence and transition to an androgen-independent state [60]. Another MAO inhibitor, clorgyline, has shown to have anti-proliferative and pro-differentiation activity on epithelial cells derived from high grade-PCa and, more recently, the γ-pyronenamoline has been described to inhibit PCa proliferation both in vitro and in vivo via KDM1A suppression [61]. A small number of KDM4 inhibitors have been developed over the past last years, but none of them has been tested in PCa. In 2010, a series of hydroxamic acids targeting KDM4A/4C has been produced and tested. In vitro, these compounds demonstrated very low effect on PCa

cells as single agents, but displayed synergistic activity in combination with the tranylcypromine analogue NCL-2 [62].

Noticeable, KDM1A and HDAC inhibitors have shown synergistic antitumor activity on glioblastoma cells [63]. The HDAC inhibitor vorinostat by inhibiting also EZH2 and H3K4 demethylases at micro-molar concentrations represents a promising epigenetic drug for cancer therapy.

miRNA and CRPC

MicroRNAs (miRNAs) are small non-coding RNA, which are approximately 19–22 nucleotides in length. In 1993 and 2000, the first two miRNAs, lin-4 and let-7, were identified in *Caenorhabditis elegans* [64, 65]. To date, there are 1,872 miRNAs that have been discovered in the human genome (miRBase: Released 20-June 2013). Furthermore, miRNAs have been shown to be involved in the regulation of cell differentiation, development, metabolism, cell cycle, and signaling transduction pathways by targeting 3′ untranslated region (3′ UTR) of target mRNA. This binding leads to degradation of target mRNAs. Dysregulation of miRNAs has been reported in infection diseases, cardiovascular diseases, neurodegenerative diseases, and cancer [66]. For this reason, numerous studies have investigated the function, underlying mechanisms, and therapeutic targeting of miRNAs in multiple disease types. Specific to CRPC, miRNAs have been demonstrated to be involved in disease progression, suggesting their utility as markers for diagnosis/prognosis, as well as novel therapeutic targets (Tables 20.7 and 20.8). MiRNA can also be divided into two categories, oncomir and tumor suppressor miRNA, according to their functions in promoting cell growth or inducing cell death.

Oncomirs

The miRNAs, which are amplified or upregulated in cancers, are called oncomirs [67]. Examples include miR-21, -125b, -221, and -222, which have been shown to be involved in CRPC development. miR-21 is an androgen receptor (AR)-regulated miRNA and has been shown to drive cell proliferation, castration resistance, metastasis, and resistance to apoptosis [68]. Ribas et al. demonstrated that miR-21 overexpression in LNCaP and LAPC4 cells mediates androgen-independent cell growth and enhances tumor growth following the castration in vivo, suggesting that the expression of miR-21 is sufficient to drive CRPC development [69]. Moreover, two clinical studies displayed the elevated miR-21 levels in serum and plasma of CRPC patients [70, 71], predominantly in docetaxel-resistant patients [71]. In addition, miR-21 is also regulated by hypoxia in colon and breast cancer. Liu et al. displayed that miR-21 is able to induce tumor angiogenesis in DU145 cells through enhancing HIF-1α and VEGF expression [72]. As a result, the role of miR-21 in CRPC development involves in multiple mechanisms. miR-125 is another oncomir involved in prostate tumorigenesis and androgen independency [67]. Shi et al. suggested that miR-125b promotes cell proliferation of LNCaP and LNCaP-cds1 cells, an androgen-independent cell line. Further, they exhibited that miR-125b increases tumor burden in castrated animals by targeting BAK1, PUMA, and p53, which are three pro-apoptotic genes [73–75]. Schaefer et al., however, displayed the downregulation of miR-125b in metastatic prostate cancer patients. Further investigations of miR-125b in CRPC are still needed. miR-221 and -222 are both from the same cluster on chromosome X and regulated by AR. Sun et al. indicated that both miRNAs are upregulated in androgen-independent cell lines, C4-2B and LNCaP-abl. Ectopic expression of miR-221 and -222 in LNCaP cells is able to reduce the dependency of androgen sensitivity. Moreover, they concluded that the overexpression of both miRNAs in LNCaP significantly induces cell proliferation by targeting *CDKN1B*, *RAB1A*, and *HECTD2* [76, 77]. Importantly, they identified the significantly high expression levels of miR-221 and miR-222 in CPRC tumors [78]. As a result, miR-221 and -222 may play an important role in CRPC progression through suppressing *RAB1A* and *HECTD2* expressions [77].

Table 20.7 The in vitro studies of miroRNAs in prostate cancer

MicroRNA	Role in PCa	Cell lines	Targets	Functions	Reference
miR-20a	Oncomir	PC3	E2F1-3	Apoptosis	[82]
miR-21	Oncomir	LNCaP; LAPC4	PTEN; AKT; Androgen pathway	mTOR pathway; Androgen independency	[69]
miR-27a	Oncomir	LNCaP	Prohibition	Androgen receptor pathway	[96]
miR-32	Oncomir	LNCaP	BTG2	Apoptosis	[83]
miR-106	Oncomir	LNCaP	p21	Cell cycle control	[97]
miR-125	Oncomir	PC3; DU145; PC-346C; LNCaP; LNCaP-cds2; LNCaP-R273H;	BAK1; PUMA; p53	Apoptosis	[75]
miR-141	Oncomir	LNCaP; VCaP; LAPC4; PC3; DU145; 22Rv1	p27	Apoptosis	[98]
miR-221	Oncomir	LNCaP; LNCaP-abl	p27; HECTD2; RAB1A	Cell cycle control and Androgen independency	[76]
miR-222	Oncomir	LNCaP; LNCaP-abl	p27; HECTD2; RAB1A	Cell cycle control and Androgen independency	[76]
miR-1	Tumor suppressor	PC3; RWPE-1; LNCaP; 22Rv1	F-actin	Cellular organization	[99]
miR-15a-16 cluster	Tumor suppressor	RWPE-1; LNCaP	Bcl2; Cyclin D; WNT3A	Cell cycle control and apoptosis	[100]
miR-23b/-27b	Tumor suppressor	ALVA31; LNCaP; PC3-ML	Rac1	Metastatic processes	[101]
miR-31	Tumor suppressor	RWPE-1; PC-3; LNCaP; 22Rv1; VCaP; DU145	E2F6	Apoptosis and chemoresistance	[102]
miR-34a	Tumor suppressor	PC3	SIRT1	Cell cycle control and chemo resistance	[91]
miR-101	Tumor suppressor	DU145	EZH2	Gene expression; proliferation	[92]
miR-143	Tumor suppressor	DU145; PC3	K-RAS; pERK1/2; cyclin D	Cell proliferation, migration, and chemosensitivity to docetaxel	[103]
miR-145	Tumor suppressor	PWR-1E; PC3; LNCaP; DU145	TNFSF10	Apoptosis	[104]
miR-146a	Tumor suppressor	DU145; PC3	ROCK1	Cell proliferation, migration, and tumorigenicity	[105]
miR-200 family	Tumor suppressor	PC3	ZEB1; ZEB2; Snail2	EMT	[106]
miR-205	Tumor suppressor	RWPE-1; PC-3; LNCaP; 22Rv1; VCaP; DU145	Bcl-w	Apoptosis and chemoresistance	[102]
miR-449	Tumor suppressor	RWPE-1; PC-3; LNCaP; DU145	HDAC1	Cell proliferation and gene expression	[107]

Table 20.8 MicroRNA expression levels in CRPC patients

MiRNA	Expression level	Sample amount	Resource	Method	Significance	Reference
let-7f	Down	15	Prostatectomy	qRT-PCR	$p=0.0286$	[88]
miR-15a	Down	17	Bone marrow	qRT-PCR	$p<0.001$	[78]
miR-16	Up	25	Plasma	qRT-PCR	$p<0.01$	[70]
miR-18a	Up	14	TURP	Microarray	$p<0.001$	[108]
miR-19b	Down	15	Prostatectomy	qRT-PCR	$p=0.0286$	[88]
miR-21	Up	25	Plasma	qRT-PCR	$p<0.001$	[70]
	Up	14	TURP	Microarray	$p=0.00694$	[83]
	Up	10	Serum	qRT-PCR	$p=0.016$	[71]
miR-22	Down	15	Prostatectomy	qRT-PCR	$p=0286$	[88]
miR-23b	Down	17	Bone marrow	qRT-PCR	$p<0.001$	[78]
miR-26b	Down	15	Prostatectomy	qRT-PCR	$p=0.0286$	[88]
miR-27a	Down	16	Prostatectomy	qRT-PCR	$p=0.0286$	[88]
miR-27b	Down	17	Bone marrow	qRT-PCR	$p<0.001$	[78]
	Down	15	Prostatectomy	qRT-PCR	$p=0.0286$	[88]
miR-29a	Down	15	Prostatectomy	qRT-PCR	$p=0.0286$	[88]
miR-29b	Down	15	Prostatectomy	qRT-PCR	$p=0.0286$	[88]
miR-30a-5p	Down	15	Prostatectomy	qRT-PCR	$p=0.0286$	[88]
miR-30b	Down	15	Prostatectomy	qRT-PCR	$p=0.0286$	[88]
miR-30c	Down	15	Prostatectomy	qRT-PCR	$p=0.0286$	[88]
miR-32	Up	14	TURP	Microarray	$p=0.00126$	[83]
miR-96	up	4	Serum	qRT-PCR	$p=0.0022$	[109]
miR-99a	Down	14	TURP	Microarray	$p=0.04490$	[83]
miR-99b	Down	14	TURP	Microarray	$p=0.00126$	[83]
miR-100	Down	15	Prostatectomy	qRT-PCR	$p=0.0286$	[88]
miR-124	Up	4	Serum	qRT-PCR	$p=0.025$	[109]
miR-126	Up	25	Plasma	qRT-PCR	$p<0.001$	[70]
miR-141	Up	25	Plasma	qRT-PCR	$p<0.001$	[70]
	Up	14	TURP	Microarray	$p<0.01$	[108]
	Up	25	Serum	qRT-PCR	$p<0.05$	[80]
	Up	18	Serum	qRT-PCR	$p=0.0276$	[109]
	Up	25	Serum	qRT-PCR	$p<0.0001$	[110]
	Down	15	Prostatectomy	qRT-PCR	$p=0.0286$	[88]
miR-146	Down	5	TURP	qRT-PCR	$p<0.05$	[105]
miR-148a	Up	14	TURP	Microarray	$p=0.04345$	[83]
	Down	15	Prostatectomy	qRT-PCR	$p=0.0286$	[88]
miR-151-3p	Up	25	Plasma	qRT-PCR	$p<0.001$	[70]
miR-152	Up	25	Plasma	qRT-PCR	$p<0.001$	[70]
miR-184	Up	6	Prostatectomy	qRT-PCR	$p=0.0286$	[88]
miR-198	Up	6	Prostatectomy	qRT-PCR	$p=0.0286$	[88]
miR-200a	Up	25	Serum	qRT-PCR	$p=0.007$	[110]
miR-200c	Up	25	Plasma	qRT-PCR	$p<0.001$	[70]
	Up	25	Serum	qRT-PCR	$p=0.017$	[110]
miR-203	Up	17	Bone marrow	qRT-PCR	$p=0.068$	[78]
miR-205	Up	25	Plasma	qRT-PCR	$p<0.05$	[70]
	Down	14	TURP	qRT-PCR	$p<0.001$	[80]
	Down	15	Prostatectomy	qRT-PCR	$p=0.0286$	[88]
miR-210	Up	25	Serum	qRT-PCR	$p=0.022$	[110]

(continued)

Table 20.8 (continued)

MiRNA	Expression level	Sample amount	Resource	Method	Significance	Reference
miR-221	Down	14	TURP	Microarray	$p<0.01$	[108]
	Down	14	TURP	Microarray	$p=0.00280$	[83]
	Up	17	Bone marrow	qRT-PCR	$p<0.001$	[78]
miR-222	Up	17	Bone marrow	qRT-PCR	$p<0.001$	[78]
miR-298	Up	25	Serum	qRT-PCR	$p<0.05$	[80]
miR-302b	Up	4	Serum	qRT-PCR	$p=0.0192$	[109]
miR-302c	Up	6	Prostatectomy	qRT-PCR	$p=0.0286$	[88]
miR-345	Up	6	Prostatectomy	qRT-PCR	$p=0.0286$	[88]
miR-346	Up	25	Serum	qRT-PCR	$p<0.05$	[80]
miR-375	Up	25	Plasma	qRT-PCR	$p<0.001$	[70]
	Up	14	TURP	Microarray	$p<0.05$	[108]
	Up	25	Serum	qRT-PCR	$p<0.05$	[80]
	Up	25	Serum	qRT-PCR	$p<0.0001$	[109]
	Up	25	Serum	qRT-PCR	$p=0.009$	[110]
miR-378	Up	13	Serum	qRT-PCR	$p=0.0057$	[109]
miR-409-3p	Down	24	Serum	qRT-PCR	$p=0.0297$	[109]
miR-423-3p	Up	25	Plasma	qRT-PCR	$p<0.001$	[78]
miR-489	Up	8	Serum	qRT-PCR	$p=0.0466$	[109]
miR-491	Up	6	Prostatectomy	qRT-PCR	$p=0.0286$	[88]
miR-513	Up	6	Prostatectomy	qRT-PCR	$p=0.0286$	[88]
miR-520d-5p	Up	26	Serum	qRT-PCR	$p=0.414$	[109]
miR-548a-3p	Up	25	Serum	qRT-PCR	$p=0.0302$	[109]
miR-548c-3p	Up	25	Serum	qRT-PCR	$p=0.0216$	[109]
miR-590-5p	Up	14	TURP	Microarray	$p=0.00356$	[83]
miR-623	Down	15	Serum	qRT-PCR	$p=0.0362$	[109]
miR-875-5p	Up	20	Serum	qRT-PCR	$p=0.0287$	[109]
miR-892b	Up	8	Serum	qRT-PCR	$p=0.004$	[109]

miR-141 has been indicated as a promising prostate cancer biomarker, which is 46-fold upregulation in prostate cancer [79]. Two independent laboratories displayed the elevated levels of miR-141 in metastatic CRPC patients [70, 80]. Two other oncomirs, miR-20a and miR-32, are involved in cell cycle regulation and resistance to apoptosis [79]. miR-20a is the member of miR17-92 cluster, which is directly activated by c-MYC, and overexpressed in prostate cancer samples [81]. Sylvestre et al. showed that miR-20a enhances the cell growth and prevents apoptosis by inducing E2F1, 2, and 3 expressions [82]. miR-32 is regulated by androgen and targets the tumor suppressor, *BTG2*, and has been demonstrated to be elevated in tumor samples of CRPC patients, resulting in the *BTG2* reduction and shorter progression-free survival rate [83].

Tumor Suppressor miRs

MiRNAs, which are downregulated or deleted in cancers, are called tumor suppressor miRNAs. These miRNAs are suggested to repress oncogene functions to induce cell cycle arrest, apoptosis, loss of invasion, and metastatic ability. In cancer cells, however, they are epigenetically regulated or deleted, leading to tumorigenesis [67]. Several tumor suppressor miRNAs have been shown in numerous cancers, such as let-7, miR-15/16, miR-34, miR-101, miR-143/145, and miR-200 family [66, 84]. Let-7c belongs to let-7 family, which encodes 13 homologous miRNAs located in the genomic locations that are frequently lost in cancers [85]. Nadiminty et al. revealed that let-7c down regulates AR transcription and translation resulting in repression of

LNCaP, C4-2B, and DU145 cell proliferation. In a cohort of prostate cancer samples, they indicated that the expression level of let-7c is negatively correlated with AR. They also demonstrated that ectopic expression of let-7c is able to prevent C4-2B and DU145 tumor growth in vivo [86, 87]. Moreover, two studies displayed the loss of let-7c in prostate cancer tumor samples by miRNA array and real-time PCR analysis, suggesting the tumor suppressor function of let-7c in prostate cancer development [88, 89]. miR-34 is a p53-induced microRNA, which is markedly activated by DNA damage and oncogenic stress. Its suppression has been shown in several cancers that also are devoid of p53 expression [79, 90]. Overexpression of miR-34 represses cell growth and self-renewal capacity in various prostate cancer cell lines by repressing AR, PSA, and Notch-1. Fujita et al. also demonstrated that ectopic expression of miR-34 in a p53-defective cell line, PC3, is capable of increasing the sensitivity to camptothecin, suggesting that miR-34 could be a marker predicting the efficacy to chemotherapy [91]. miR-15a and miR-16-1 are from the same cluster on chromosome 13q14, and is frequently down regulated in prostate cancer and correlated with tumor progression. Bonci et al. revealed that these two miRNA are lost in 16 of 20 prostate cancer patients. miR-15 and miR-16-1 target various oncogenes, such as *BCL2*, *CyclinD*, *WNT3A*, *VEGF*, *IL-6* to reduce cell proliferation, invasion, and angiogenesis. Watahiki et al. displayed the significant reduction of miR-16 in metastatic CRPC patients, suggesting that loss of miR-15a or miR-16-1 might be a marker to understand prostate cancer progression [70, 79]. EZH2, a histone methyltransferase, has been illustrated to be a critical mediator of progression toward CRPC and regulated by miR-101 [45]. Varambally et al. indicated that the loss of miR-101 happened in six of 17 localized prostate cancer and 25 of 33 metastatic prostate cancer patients, suggesting that miR-101 may be a potential marker in understanding disease progression [79]. Furthermore, they demonstrated that ectopic expressing miR-101 in DU-145 cells results in reduced anchorage-independent cell proliferation and slower tumor growth in vivo, indicating the tumor suppressor function of miR-101 [92].

Epithelial–mesenchymal transition (EMT) is a process that tumor cells gain the characteristics of mesenchymal cells to have greater mobility and invasive ability, leading to metastatic disease. Several tumor suppressor miRNAs have been identified not only to control cell growth, but also importantly, regulate EMT. miR-200 is a member of miR-200 family, which interrupts the EMT pathway by repressing ZEB1 and ZEB2 function [93]. Kong et al. displayed that the expression level of miR-200 in PC3-PDGF-D expressing cells, which are prostate cancer cells with high metastatic potential, is downregulated. Also, they suggested that miR-200 is able to eliminate stem-like cells by repressing Notch-1 and lin-28B pathways [94]. Two other metastasis repressor miRNAs are miR-143 and -145. Peng et al. exhibited that these two miRNAs are reduced in 13 bone metastasis tissues comparing to 16 primary prostate tumors. Further, they indicated that ectopic expression of miR-143 and -145 in PC3 cells can prevent bone metastasis in animals, suggesting the role of miR-143 and -145 in metastatic disease [95].

MiRNA is a compelling field in cancer research. Numerous miRNAs have been identified in human genome regulating physiological functions and disease progression. Although researchers have illustrated the miRNA profile in castration resistant prostate cancers and suggested for diagnostic and prognostic markers, there are still many controversies. For example, miR-222, which has been suggested to be an oncomir, has been also reported to be decreased in patients [88, 111]. This might be explained by different techniques and specimen collections. For this reason, new advanced techniques are needed to provide more precise information and apply on CRPC therapies.

Conclusions

Epigenetics represents a new field for therapeutic interventions in cancer, including CRPC. Several key enzymes regulating gene expression involved in tumorigenesis have been identified and are “druggable” targets. The preclinical data on the role of HDACs, HMTs, DMs, and MIRs in

CRPC offer a scenario for a complex but promising drug development program. The clinical success of epigenetic therapies for CRPC in rational combination strategies will be dictated by the identification of the patients who might be most suitable for this therapeutic approach.

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Undifferentiated Prostate Cancer and the Neuroendocrine Phenotype

21

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Introduction

The development and differentiation of the normal prostate gland as well as prostate cancer is regulated by androgen receptor (AR) signaling. Consequently, androgen deprivation therapy (ADT) remains the standard treatment for patients with advanced prostate cancer. Initially tumor regression is seen in the majority of patients, but unfortunately over a period of time, typically 1–2 years, disease progression occurs with return of AR signaling activity and a transition to a castration-resistant state. Despite castrate levels of serum testosterone, a critical step in the development of castration-resistant prostate cancer (CRPC) is reactivation of AR signaling [1–3]. A number of potential mechanisms important for AR reactivation include constitutive activation of the AR through gene amplification, alternative gene splicing [4], AR-activating gene mutations, intra-tumoral production of androgen, promiscuity of the AR with binding of alternative steroid hormone ligands, activation of downstream targets through dysregulation of transcription factors [5], and other yet unidentified mechanisms. This has served the basis for the clinical development and FDA approval of highly potent AR signaling-targeted drugs for the treatment of patients with metastatic CRPC [5, 6]. However, in a subset of patients, CRPC may escape the need for androgen signaling and evolve into an undifferentiated, AR negative or AR low tumor, which may or may not display features of neuroendocrine differentiation (NED). These tumors are often referred to as neuroendocrine prostate cancer (NEPC) or anaplastic prostate cancer.

The diagnosis of NEPC or anaplastic prostate cancer is usually based upon the presence of certain distinctive clinical features such as low or moderately elevated serum prostate-specific antigen (PSA), predominantly visceral or lytic bone metastases, elevated serum markers of NED [such as chromogranin A (CgA), Neuron specific enolase (NSE)], and/or other clinical features suggestive of aggressive hormone independent disease (Table 21.1). Biopsy in this setting can result in a spectrum of histologies, ranging from poorly differentiated or undifferentiated carcinoma, mixed adenocarcinoma-NEPC, pure small cell carcinoma, or large cell neuroendocrine carcinoma. Immunohistochemistry for neuroendocrine markers is often performed but is not always positive in patients with suspected NEPC or anaplastic clinical features and no standard definition of % of positive tumor cells exists. Therefore, there are many challenges that remain in the recognition and management of this disease phenotype.

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F. Saad and M.A. Eisenberger (eds.), *Management of Castration Resistant Prostate Cancer*, Current Clinical Urology, DOI 10.1007/978-1-4939-1176-9_21,

Table 21.1 Clinical features of "anaplastic" prostate carcinomas (eligibility criteria for study entry)

Castrate-resistant[a] *prostate carcinoma with at least 1 of the following:*
C1. Histologic evidence of small-cell prostate carcinoma (pure or mixed)
C2. Exclusively visceral metastases
C3. Radiographically predominant lytic bone metastases by plain x-ray or CT scan
C4. Bulky (≥5 cm) lymphadenopathy or bulky (≥5 cm) high-grade (Gleason ≥ 8) tumor mass in prostate/pelvis
C5. Low PSA (≤10 ng/mL) at initial presentation (before ADT or at symptomatic progression in the castrate setting) plus high volume (≥20) bone metastases
C6. Presence of neuroendocrine markers on histology (positive staining of chromogranin A or synaptophysin) or in serum (abnormal high serum levels for chromogranin A or GRP) at initial diagnosis or at progression. Plus any of the following in the absence of other causes: **A**. elevated serum LDH (≥2×IULN); **B**. malignant hypercalcemia; **C**. elevated serum CEA (≥2×IULN)
C7. Short interval (≤6 months) to androgen-independent progression following the initiation of hormonal therapy with or without the presence of neuroendocrine markers

GRP gastrin-releasing peptide
[a]Patients with small-cell prostate carcinoma on histologic evaluation were not required to have castrate-resistant disease

Neuroendocrine (NE) Cells of the Normal Prostate

The epithelial compartment of the normal prostate gland consists of three types of epithelial cells—basal (proliferating), secretory (luminal), and NE cells interspersed within the basal cells. Neuroendocrine cells constitute <1 % of all the epithelial cells and are terminally differentiated. Therefore, NE cells in normal prostate typically lack proliferative activity and do not express the proliferation associated Ki-67 (MIB-1) antigen. Neuroendocrine cells are also AR negative, and thus do not express downstream AR target proteins including PSA [7–9]. Cellular extensions of NE cells in the form of neurite-like projections can help establish communication between NE cells and surrounding epithelial cells [10, 11]. This allows for effective paracrine signaling including secretion of products from a wide range of neurosecretory granules present in NE cells, including serotonin, histamine, CgA, calcitonin gene family of peptides (calcitonin, katacalcin, and calcitonin gene-related peptide), neuropeptide Y, vasoactive intestinal peptide, bombesin, gastrin-releasing peptide (GRP), parathyroid hormone-related protein (PTHrP), NSE, thyroid-stimulating hormone-like peptide, vascular endothelial growth factor, and others [11]. This paracrine signaling is important in regulating the growth, survival, and differentiation of the surrounding normal prostate epithelial cells in an androgen-independent manner. Neuroendocrine cells can be isolated in normal prostate by specific immunostaining with antibodies against NSE, CgA, and synaptophysin [12, 13] and may be more prevalent in African Americans [14].

NED in Prostate Cancer

In clinically localized prostate adenocarcinoma, NE cells are typically focally distributed as single cells or in small nests amongst a predominant population of malignant prostate epithelial cells. On average, NE cells tend to constitute no more than 1 % of all tumor cells, except for the rare cases of small-cell prostate carcinoma or prostate carcinoid. The World Health Organization (WHO) has categorized NED in prostate cancer into three forms: (1) focal NED in conventional prostate adenocarcinoma; (2) carcinoid tumor (well-differentiated NE tumor); and (3) small cell NE carcinoma (poorly differentiated NE carcinoma) [15]. Depending upon the type of the detection methods utilized, the prevalence of NED in prostate cancers varies from 10 to 100 % and may carry prognostic significance [13, 16–20].

The amount of NED seen in prostate cancer increases with disease progression and with castration resistance (Fig. 21.1). Similarly, increased expression of CgA in the prostate and serum of patients on anti-ADT is commonly seen with disease progression [17]. Biopsies during stages of prostate cancer progression may often detect poorly differentiated tumors or those with mixed features containing both epithelial and neuroendocrine features. In a small subset of patients, transformation to predominantly androgen-independent

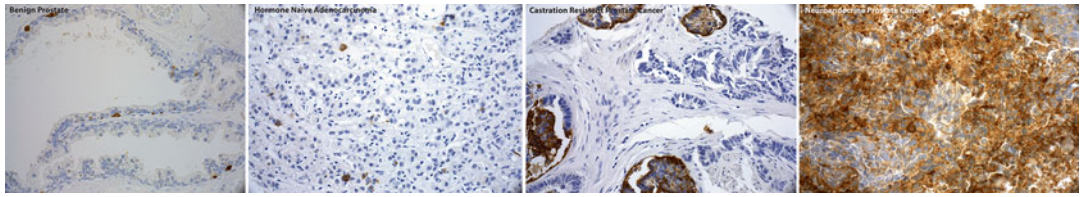

Fig. 21.1 Immunohistochemistry for Chromogranin showing representative examples of the spectrum of prostate cancer progression: Chromogranin IHC is negative in benign prostate (*first panel*), focally positive in hormone naïve prostate cancer (*second panel*), tends to increase in CRPC (*third panel*), and shows diffuse positive staining in NEPC (*fourth panel*)

NEPC can occur. At this transition, AR expression may be low or absent. This is often associated with clinical features of anaplastic prostate cancer (Table 21.1). The development of the clinically defined anaplastic phenotype predicts poor prognosis and response to platinum-based chemotherapy [21]. These tumors often are poorly differentiated, lack AR expression, and may show pure small cell or predominantly NE features.

Histology

In the setting of CRPC, NEPC typically refers to the presence of small cell carcinoma histology on tumor biopsy. Small cell carcinoma of the prostate consists of sheets and nests of small, rather uniform cells, with nuclear molding and scant cytoplasm. Nuclei are rounded and hyperchromatic, containing fine chromatin pattern and inconspicuous nucleoli. Mitoses are numerous, occurring at a rate of 5–10 per high-power field. Tumor necrosis is also common. The tumor tends to infiltrate widely and diffusely without well-defined margins. Lymphatic and blood vessel invasion are frequent. Large cell neuroendocrine carcinoma can also be seen but this is an extremely rare variant of NEPC. Large cell NEPC has an architecture that suggests NED. The tumor is composed of sheets and ribbons of large and polygonal cells with abundant, often eosinophilic, cytoplasm and nuclei with coarse chromatin and frequent nucleoli. The mitotic rate is high and necrosis is usually prominent. Rosette-like structures can be observed [22]. In approximately 50 % of cases, NEPC tumors are mixed with conventional adenocarcinoma [23, 24].

Today, the diagnosis of NEPC is primarily based on morphology but immunohistochemistry may be helpful in supporting the diagnosis**.** Neuroendocrine cancer cells are typically positive for one or more neuroendocrine markers such as synaptophysin, NSE, CgA, CD56, and are generally negative for AR and androgen-dependent markers such as PSA, prostatic acid phosphatase (PAP), and ETS-related gene (ERG). NEPC is negative for all neuroendocrine markers in approximately 10 % of cases. The diagnosis of metastatic NEPC may be challenging. In the case of a poorly differentiated metastatic adenocarcinoma, history of primary prostate cancer treated by long-term ADT, with early visceral metastasis and/or a normal or slightly elevated serum PSA level, clinicians should consider immunohistochemistry staining to evaluate for NEPC. In a patient that presents with small cell carcinoma of unknown primary, distinguishing prostatic small cell carcinoma from small cell carcinoma of another origin can also be challenging as they can appear morphologically similar and can both express common NE markers. Testing for the presence of the prostate-specific ERG gene fusion by FISH, which occurs in approximately 50 % of NEPC, can help confirm prostate origin and excludes neuroendocrine carcinoma from other primary sites [25, 26].

Molecular Mechanism of NEPC

The mechanisms and pathways that lead to NED in prostate cancer and the triggers of transformation to an AR low or AR negative, poorly differentiated or neuroendocrine phenotype in a patient

with known adenocarcinoma of the prostate are poorly understood. It is believed that a synergistic functional network of pathways, rather than a single pathway is involved in the process of induction and sustenance of NED [27]. Androgen withdrawal is the most potent stimulator of NED in LNCaP prostate cancer cells, however various cytokines have also been reported to induce NED [28–32]. Neuroendocrine cells may also establish paracrine networks within the tumor and stimulate the proliferation of adenocarcinoma, invasion, metastasis, and progression to a castration-resistant stage. This likely is mediated through increased expression of receptors on the bulk non-NE/adenocarcinoma tumor cells for neuropeptides, biogenic amines, and cytokines secreted by the NE cells [10, 11, 33].

The addition of the cytokines interleukin-8 (IL-8) or interleukin-6 (IL-6) can induce NED in LNCaP cells by activating downstream signaling pathways involved in signal transduction, activation of transcription, mitogen-activated protein kinases, cyclic adenosine monophosphate-dependent protein kinase [34]. IL-6 can also activate phosphatidylinositol-3-kinase (PI3K)-dependent signaling pathways [35–37]. Activation of ERK and PI3K-AKT-mTOR signaling pathways along with androgen deprivation has also been reported to induce NED in LNCaP cells [38]. Cortes et al. reported that when LNCaP cells were treated with epidermal growth factor (EGF) in the presence of LY294002, an inhibitor of the PI3K-AKT pathway, an increase in the levels and activity of ErbB2 is observed. This finding was associated with cell survival and NE differentiation.

Overexpression of CXCR1, a receptor for IL-8, has been implicated in CRPC progression. CXCR2, which is homologous to CXCR1, is exclusively expressed on NE tumor cells. Normally the IL-8/CXCR2/P53 signaling pathway keeps the NE tumor cells in a quiescent state in an autocrine manner [39], and TP53 is critical in mediating this pathway. Loss of TP53 and/or other mechanisms resulting in dysregulation of the IL-8/CXCR2/P53 signaling pathway may represent a mechanism for NE cell proliferation [40]. Knockdown of TP53 using siRNA abolishes the growth inhibition of both LNCaP/CXCR2 and PC-3 cells by IL-8 [40, 41]. The role of TP53 in NEPC is further supported by the transgenic adenocarcinoma of mouse prostate (TRAMP) model, in which small cell prostate cancer develops as a result of SV40 T antigen inactivation of both TP53 and Rb. Similarly, Nikitin et al. developed a $TP53^{-/-}Rb^{-/-}$ double knockout mouse model in which probasin promoter was used to drive the expression of the Cre-recombinase, and the resultant tumors exhibit a morphology similar to NEPC [42].

Recently, other genes and pathways have been implicated in the development of NEPC. Amplification of the cell cycle kinase, Aurora Kinase A (AURKA), and the transcription factor and oncogene, N-myc (MYCN) has been detected in NEPC tissues and thought to functionally cooperate to induce NEPC [43]. Importantly, this is potentially targetable using a small molecule aurora kinase inhibitor [43]. Loss of the REST transcriptional complex is another potential key driver in the development of NEPC [44]. Midkine (MDK) is a retinoic acid-induced heparin binding growth factor, highly expressed during embryogenesis is involved in neurogenesis and epithelial-to-mesenchymal transition (EMT). Its expression has been correlated with poor clinical outcomes in various human cancers, including prostate cancer [45–49]. Immunohistochemical analysis of MDK, the neuronal marker tubulin-beta III (TUBB3), and the NE-marker CgA was performed in 53 patients with PCa (hormone naïve and CRPC). Up-regulation of MDK, TUBB3, and CgA was observed in CRPC compared to hormone-naïve tumors. MDK expression was highly associated with the expression of both CgA and TUBB3, with MDK positive NE-like looking cells co-expressing CgA or, more commonly, CgA together with TUBB3. It was proposed that MDK up-regulation in CRPC is associated with NED (shown by its relation to CgA and TUBB3) and hypothesized as a potential target for prostate cancer therapy [50].

Clinical Presentation

Patients rarely present with de novo NEPC (<1 %), most commonly in form of small cell prostate cancer, and typically associated with metastatic disease at initial presentation. Unlike prostate adenocarcinoma, NEPC can often present with visceral metastasis (liver, brain) and/or lytic bone lesions. It can be challenging to distinguish NEPC from small cell carcinoma of other primary sites as they have similar histological and immunohistochemical features, i.e., absence of androgen-regulated genes and presence of neuroendocrine markers. In this case, determination of TMPRSS2-ERG gene rearrangement by FISH may prove helpful as it is present in about 50 % of prostate cancers but is universally absent in small cell cancers from other primary sites [25, 26]. Uncommonly, NEPC may also present with constitutional symptoms, hydronephrosis, bone pain, abdominal pain, hematochezia, or hematuria. Ectopic production of hormones such as adrenocorticotrophic hormone, antidiuretic hormone (ADH), thyroxine, etc., by the tumor may occasionally present as a paraneoplastic syndrome, with clinical manifestations of thyrotoxicosis, inappropriate ADH production, hypercalcemia, and/or adrenal hyperfunction. About 10 % of the patients with small cell NEPC present with paraneoplastic syndromes. Rarely, NEPC is included in multiple endocrine neoplasia 2A (Sipple syndrome), including malignant pheochromocytomas, thyroid medullary carcinomas, and parathyroid hyperplasia [51].

The role of NED markers in primary prostate adenocarcinoma as prognostic indicators is controversial. Some studies have found an association between NED and a worse prognosis [52–54], whereas others failed to find this relationship [55, 56]. There is a correlation between the immunohistochemically detected CgA and the serum levels of CgA [55, 57]. Serum CgA and NSE levels appear to be a useful indicator for detection of NED in patients with CRPC [58]. And elevated levels at earlier stages of the disease may indicate impending resistance to hormone therapy [59]. Following levels of serum markers such as CgA, NSE while on therapy has not shown correlation with response to therapy [57]. Carcinoembryonic antigen (CEA), a non-prostate-specific tumor marker [60, 61], is also often elevated in a subset of NEPC [62]. However, no obvious correlation has been detected between serum CEA levels and the levels of PSA and other NE markers.

Treatment

Despite being sensitive to both chemotherapy and radiotherapy similar to small cell lung cancer, most patients with NEPC or anaplastic prostate cancer only have a short-term response and eventually die within 1 year of diagnosis due to disease progression [63, 64]. Introduction of chemotherapy at early stages has shown some palliative benefit and a modest survival advantage. In one older study, a survival of 2 years in 20 % of patients was reported in patients receiving chemotherapy [65]. Rarely, transient remissions have been reported in patients who have received multimodality therapy with chemotherapy, surgery, and radiation.

As very few patients with NEPC present with localized disease at initial presentation, there is limited data on management of patients who present with organ confined disease. These patients are very likely to have occult metastatic disease not detected on bone scan or computerized tomography. Positron emission tomography (PET) may be useful in confirming localized disease. It is recommended to use a multimodality approach in treating these patients similar to small cell lung cancer, consisting of chemotherapy with concurrent or consolidative radiotherapy to control the local disease as well as systemic micro-metastasis [66]. Very limited data is available in context of use of surgical approach in this clinical setting.

Most patients with advanced NEPC or anaplastic prostate cancer have tumors with variable histologies or mixed features with both NEPC and prostate adenocarcinoma components. Therefore it is rational to start with ADT. This approach will treat any androgen responsive

cancer while chemotherapy will treat the NEPC component which is typically not responsive to androgen deprivation. Platinum-based chemotherapy regimens, most commonly a regimen of cisplatin and etoposide similar to what is used in small cell lung cancer, have demonstrated major responses in NEPC. Other agents such as ifosfamide and doxorubicin have also shown antitumor activity. In 2002, Papandreou et al. reported a phase II clinical trial with doxorubicin, cisplatin, and etoposide in 38 patients with fully characterized small cell carcinoma of prostate. A response rate of 61 % with no complete response was reported. However, no improvement in the median time to progression (5.8 months) and overall survival (10.8 months) was observed, instead the response was associated with greater toxicity as compared to cisplatin and etoposide alone [63]. In 2011, Flechon et al. reported the results of phase II study with carboplatin and etoposide in 60 patients. It was concluded that the benefit-risk ratio of the regimen seems unfavorable due to the poor response and high toxicity [64]. Most recently, Aparicio et al. prospectively defined seven clinical features that were considered characteristic of NEPC or anaplastic prostate cancer to select patients for a single-arm sequential, 120 patient, phase II clinical trial (C1–C7; Table 21.1). Men who met one or more of these criteria were treated with carboplatin and docetaxel (CD), and with etoposide and cisplatin (EP) upon progression. 65.4 and 33.8 % of patients were progression free after four cycles of CD and EP, respectively. Median overall survival (OS) was reported as 16 months [95 % confidence interval (CI), 13.6–19.0 months]. Bulky tumor mass was significantly associated with an inferior outcome. Lactic acid dehydrogenase (LDH) strongly predicted for OS and rapid progression. Serum CEA concentration strongly predicted OS but not rapid progression. Neuroendocrine markers did not predict outcome or response to therapy [21]. As amplification of AURKA and MYCN has shown to induce NED, currently a phase II trial with AURKA inhibitor, MLN8237 (Alisertib) is underway to identify response with this potential therapy in NEPC patients.

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Part V

Clinical Management Aspects

22 Strategies Addressing Quality of Life: Management of Patient-Reported Outcomes and Symptoms

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Introduction

Prostate cancer is the first cancer for which a therapy was approved based on a patient-reported outcome (PRO) alone with no change in survival, setting an example for a new wave of patient-based measures that fit with patient-centered care. In the 1990s, Dr. Ian Tannock and colleagues randomized 161 patients with castrate-resistant prostate cancer (CRPC) to mitoxantrone intravenous infusion plus prednisone pills versus prednisone alone [1]. They observed no significant difference in overall survival, but the group that received mitoxantrone had significantly increased global quality of life (QOL) ($p = 0.009$) and concordant improvements in several areas including physical functioning. The pain control lasted 43 weeks versus just 18 weeks with prednisone alone [2]. Dr. Tannock and his colleagues used a relatively new approach, completely novel to most oncologists, to measure QOL by actually asking the patients about their experience. The patients then completed forms that would eventually become familiar to oncologists—the European Organization for Research and Treatment of Cancer Quality-of-Life Questionnaire C30 [3] (EORTC QLQ-C30, a global measure applicable to all cancers), and the Quality of Life Module-Prostate 14 (QOLM-P14, which was developed to measure impact specifically in prostate cancer patients, since they have different symptoms than breast cancer patients or leukemia patients). The PRO trial data allowed investigators to show that mitoxantrone not only improved QOL but also saved money by preventing hospitalizations [4]. Based on this trial, and a similar trial performed in the United States [5], the Food and Drug Administration approved the drug with no overall survival benefit [6], just a remarkable change in QOL, ushering in a new era of enthusiasm about the treatment of prostate cancer with chemotherapy.

What are PROs, why are they important, and how do those treating CRPC patients incorporate them into clinical trials and day-to-day practice? We will start with an explanation of PROs, illustrate what is currently known about their use in various facets of treatment, and then make recommendations for future use. We will not provide an exhaustive review of the literature but rather attempt to highlight the PROs and PROMS that a clinician might encounter, and how to evaluate them.

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F. Saad and M.A. Eisenberger (eds.), *Management of Castration Resistant Prostate Cancer*, Current Clinical Urology, DOI 10.1007/978-1-4939-1176-9_22,

PROs and PROMs

PROs and PROMs (patient reported outcome measures) represent an attempt to capture the patient experience that is often missed with response rates, toxicity data, and survival figures. For example, does chemotherapy with docetaxel and its attendant fatigue and neuropathy and risk for sepsis improve pain and other prostate cancer symptoms enough to justify its use when no one is cured? If half the patients were cured, toxicity and symptoms would be relatively unimportant as in testicular cancer. But, if patients only live 2–3 months longer, does the improvement in symptoms—from the perspective of the patient, not the doctor or researcher—justify use? The answer, now well known, is "yes" because the improved survival was also associated with better QOL in 22–23 % of docetaxel patients versus just 13 % in control patients [7], again paving the way for FDA of docetaxel based on survival and PROM improvement [8]. In fact, before the modern era of drugs, randomized trials showed more difference in health-related quality of life (HRQOL) than they did in anti-cancer responses [9].

The only way to know the patient experience is to ask patients, but this process just began in the early 1990s. In 20 years we have moved from no PRO data to PRO data being an essential component of every new drug or treatment trial. The one thing all PROs have in common is that they ask the patient by questionnaire about *their own experience in terms understandable to patients*. This is very different from the toxicities we gather with such as tools as the NCI Common Terminology Criteria for Adverse Events (CTCAE) [10] (referred to often as the Common Toxicity Criteria), or typical response rates, progression free survival, or overall survival. The NCI-CTCAE measurements good validity between scores for the same questions and items, but do not directly assess symptoms [11]. Most patients want to know what will happen to them, in understandable terms. Telling them "You will likely have neuropathy but only Grade 1 or 2" (evaluation with NCI-CTCAE) is less informative than "Most patients had some nerve problems, usually numbness, but almost all were able to hold a pencil and drive a car" (Evaluation with CIPN-20 [12], part of the EORTC QLC-30 [13].)

Since PROs began as gradable forms usually filled out by patients in the waiting room, these measures have evolved and are now recorded using hand-held computers and tablets, as well as online queries using Computer Adaptive Testing (CAT) that changes the questions based on responses. The NCI-CTCAE was one of the first instruments to be tested, with electronic patient-entered data in order to have real-time PROs [14]. There are both advantages and disadvantages to real-time monitoring of PROs. The advantage is that they have high validity and reproducibility; the disadvantage is that these are important data that require monitoring. If someone reports—electronically—that they have severe pain, vomiting, or even fever and chills at 02:00 a.m., these are serious medical events that could lead to morbidity and even death. The organization that uses real-time PROs has to evolve a mechanism to manage the answers in a timely manner as circumstances dictate, just like with telephone calls [15].

Existing data do show that when symptoms are electronically reported to the clinicians, more clinical actions are taken. The cancer center at McMaster University in Ontario instituted universal electronic screening with the Edmonton Symptom Assessment Scale (ESAS) [16]. With 912 patients evaluable, the percentage of patients with a documented clinical action for pain or dyspnea increased with the severity of self-reported symptoms (from none to severe pain, 36.9 %, 49.2 %, 55.2 %, and 71.4 %; $p < .001$); however, less than half of "severe" scores prompted a documented clinical action.

Patients are interested and able to do PROMs no matter what the form. The National Institutes of Health (NIH) has developed the Patient-Reported Outcomes Measurement Information System (PROMIS) to serve as be a universal PRO, as part of the Roadmap Initiative [17]. Data from randomized cross-over trials showed no difference in the use of interactive voice response (IVR)

technology, paper questionnaire, personal digital assistant, or personal computer (PC) on the Internet, and a second form by PC [18]. Patients and families, referred to as stakeholders, have great interest in these instruments with 93 % saying that PROMIS would add benefit and could be done.

PRO instruments can be "global," attempting to measure the impact of treatment on the entire perspective of the patient, or specific to one symptom or disease. Some of the first instruments were deliberately global to attempt to capture the entire patient experience broken down into "domains" or groups of questions about physical symptoms, psychological health, sexual function, emotional distress, spiritual adaptation, etc. The term "construct" is often used in place of a characteristic, so a "single-construct instrument" will measure something like distress or pain, and a "multiple-construct instrument" will attempt to measure the impact on the whole health profile. Instruments such as the Medical Outcomes Study Short Form 36 and Functional Assessment of Cancer Therapy (FACT) captured all these outcomes, but were often "too global." For instance, what if a treatment improved bone pain substantially, or worsened sexual functioning, and did not change anything else? Factoring one answer into the average of 36 questions would show no benefit. Subsequent modifications included very specific modules designed to capture changes in specific aspects of patient experience such as nausea or pain or hematologic malignancy symptoms. Some general and specific instruments are shown in Table 22.1.

Coverage of symptoms by the general PROs falls into two categories (Table 22.2). The FACT, PRO-CTCAE, and QLQ-C30 measure most symptoms used in general HRQOL studies, while PROMISE and EQ-5D measure only a small subset of those symptoms but complement them with additional assessments such as physical function and satisfaction with social roles and activities.

There have been difficulties in using PROs and PROMs on both the scientific and administrative fronts. Scientifically, many trialists wanted to measure an average QOL score at the beginning of the trial on all the patients, then compare this to a score 2 months later. At first, this was done by taking the average of all the patients' scores at time zero and at 2 months, which inevitably showed no difference. This would be like taking the average of the whole group of tumor responses, rather than the changes in each individual's cancer. Better reporting and better computer entry have fixed this issue.

Another scientific issue has been defining the minimal amount of change in QOL that is clinically significant. While even small benefits in overall and progression-free survival have been used to justify drug use, the amount of "improved quality of life" has less universal acceptance. An early conference [35] agreed on common metrics: first, ask the patients what a clinically important change score would be, for example, 20 % change in pain. Second, ascertain what percentage of patients achieved that level of change. Such easily understandable and reportable metrics have allowed comparison across studies.

An additional issue has been whether to use patient scores, clinician scores, or both; and if both, in what order, and whose scores take precedence. Current data show that patient symptom scores, in addition to clinician scores, more accurately predict survival of patients than either set of scores alone [36]. In a study of 161 lung cancer patients followed for 12 months, patient-reported symptoms and QOL with the Euro-QOL 5 were complementary to clinician-reported toxicity scores using the NCI CTAEC. The clinician-reported scores were more predictive of emergency room visits and death than patient-reported scores for fatigue ($p<0.001$), nausea ($p=0.01$), constipation ($p=0.038$), and Karnofsky Performance Status ($p<0.001$), but the patient scores more accurately portrayed day-to-day function [37]. (This study also suggests that when these patient-reported symptoms rise to the level of clinical recognition they are serious and should be addressed.)

The main administrative hurdle to use of PROs and PROMs in clinical trials arises because most trials are done by pharmaceutical companies to satisfy registration (new drug approval) requirements. To date there has been only one drug approved for cancer based on a

Table 22.1 Commonly used patient-reported outcome metrics (PROMs) with uses, results, and commentary

PROM name	Use	What has it shown?	Comment	Authors or reference
General PROMs				
Medical outcomes study short-form health survey (SF-36 and SF-12)	Studies of populations with multiple diseases	Hard to show differences in global scores	Has been supplanted by newer disease-specific modules	Ware et al. [19]
EORTC QLQ-C30	Assesses quality of life in cancer patients and offers optional disease and context-specific modules with additional symptoms	Only moderate agreement with FACT [20]	Has been used in more than 3,000 studies; current version is 3.0	Aaronson et al. [3]
Patient-Reported Outcomes version of the Common Terminology Criteria for Adverse Events (PRO-CTCAE)	An attempt by NCI to develop a PROM extension to CTCAE	Not yet used widely—Pubmed search of PRO-CTCAE yields 4 articles versus 1,343 for EORTC QLQ-C30	Used in multiple clinical trials	Basch et al. [21]
Euroquol-5 (EQ-5D)	Provides a single summary index by applying weights based on the valuation of EQ-5D health states from general population samples	Measures cost utility, or the change in a unit of "utility" related to QOL per additional cost; often relied upon by European regulatory authorities in evaluating oncology therapies	Used by many pharmaceutical companies, recommended for use in cost-effectiveness analyses by the Washington Panel on Cost Effectiveness in Health and Medicine	Rabin et al. [22]
Functional Assessment of Cancer Therapy (FACT)	Assesses quality of life in cancer patients and offers optional context-specific modules with additional symptoms	FACT-Lung scores predict survival in non-small cell lung cancer treatment studies [23]	Widely used in the United States	Cella et al. [24]
PROMIS	Measures selected symptoms and HRQOL	Patients with chronic disease have poorer HRQOL than those without a diagnosis $N=21{,}113$ [25]		Cella et al. [26]
Domain-specific PROMs				
Palliative Care Outcome Scale (POS)	A PROM for patients with advanced cancer that assesses the key goals of palliative care	Used in measuring quality of care in patients who are dying		Hearn et al. [27]
EORTC QLQ-30 Pancreas Module (QLQ-PAN26)	Disease-specific QLQ-C30	Commonly used to measure quality of life in patients with pancreatic cancer		Fitzsimmons et al. [28]

(continued)

Table 22.1 (continued)

PROM name	Use	What has it shown?	Comment	Authors or reference
FACT-Prostate Cancer (FACT-P)	A 12-item prostate cancer subscale for FACT	Used to assess effectiveness of abiraterone [29]		Esper et al. [30]
Lung cancer symptom scale	8-min survey with nine patient and six observer items	Used to assess disease-specific burden such as fatigue, cough, dyspnea. QOL was not worse during pemetrexed maintenance versus placebo [31]	Being used by pharmaceutical companies to document symptom burden and the need to reduce it [32]	Hollen et al. [33]
Expanded Prostate Cancer Index Composite (EPIC-26)	Evaluates patient function and bother (urinary, sexual, bowel, and hormonal domains) after prostate cancer treatment	Gaining in popularity and used to develop symptom severity groups for assessing recovery after surgery		Wei et al. [34]

Table 22.2 Symptom coverage of commonly used patient-reported outcome metrics (PROMs)

Symptom	FACT	PROMIS	PRO-CTCAE	QLQ-C30	EQ-5D
Anorexia	X		X	X	
Anxiety	X	X	X	X	X
Constipation	X		X	X	
Depression	X	X	X	X	X
Diarrhea	X		X	X	
Dyspnea	X		X	X	
Fatigue	X	X	X	X	X
Insomnia	X	X	X	X	
Nausea	X		X	X	
Pain	X	X	X	X	X
Neuropathy	X		X	X	
Vomiting	X		X	X	

purely palliative endpoint: mitoxantrone. All others have been approved based on changes in survival, some as small as 2 weeks (median change 0.33 months) for the addition of erlotinib to gemcitabine in advanced pancreas cancer [38]. Most trials report the PRO scores separately from the main study results as a complement; of 77 recent trials in castrate-resistant metastatic prostate cancer only 18 % had PRO or tolerability companion studies [39]. For instance, sipuleucel-T improved survival by about 4 months [40], and had predictable declines in QOL during treatment due to fatigue, but by week 26 and thereafter QOL was identical between treatment and placebo groups [41]. Until the FDA changes its rules, PROs and PROMs will be an "add on" to the trials rather than the main endpoint [42].

A second administrative hurdle is related to the electronic medical record, which should be able to integrate PROs with little or no difficulty, and could then be used for comparative

effectiveness research. For instance, does cabazitaxel when given to patients with co-morbidities such as congestive heart failure have the same favorable effects as in clinical trial subjects [43]? The main questions would be what to measure and record for universal access, within the bounds of what is possible and not too expensive. A recent review [44] explored the "architecture" of such decision-making and proposed some rationality before we attempt to add patient portals with multiple instruments, none of which are standardized, to the mix. This will require adoption of one or several instruments capable of cross-talking and interpretation, with heightened privacy requirements and more expense, when the EMR vendors are trying to carve out market share. Such improvements are possible but will likely require major governmental intervention similar to "meaningful use" requirements.

Measuring Pain in CRPC

Pain is measured by nearly all PROMs, is associated with decreased survival in CRPC patients [45], can be debilitating and impair QOL [46], and, as was illustrated in the approval of mitoxantrone by the FDA mentioned earlier, can be an important endpoint defining treatment benefit in clinical trials. Measuring pain as a PRO is challenging. The FDA has raised its standards for measuring pain, describing how it determines whether PRO measures are adequate for use in clinical trials to support labeling claims [47]. Despite this published guidance, in the 17 years since approving mitoxantrone for the treatment of pain, the FDA has not allowed the inclusion of a pain endpoint in a prostate cancer drug label—despite multiple pain endpoints in FDA drug applications during this time [48]. Pivotal trials for both docetaxel and abiraterone acetate measured pain [7], but FDA concern about pain measurement techniques in those trials caused there to be no mention of pain reduction in the drug labels [49].

Without a viable consensus on a method for measuring pain, investigators develop pain questionnaires on a case-by-case basis with little regard for inter-trial comparability. However, a five-site clinical trial opened late in 2013, with approval from the FDA, to study a simple pain measurement PRO: "pain at its worst in the last 24 hours" [50]. This new measure is promising both because the trial design team includes the developer of the most commonly used pain questionnaire in oncology, Brief Pain Inventory (BPI), and because the trial has the active encouragement of the FDA. Results are anticipated in 2016.

Other PROs and PROMs That Clinicians May Encounter in Treating CRPC Patients

Although pain dominates discussions of PROs in CRPC patients, there is little agreement on other PROs most relevant to CRPC patients and treatment. Scher et al. suggest "other important PROs for consideration in trials and practice include anorexia (decreased appetite), anxiety, constipation, diarrhea, sleep disturbance, mucositis, nausea, pain, peripheral sensory neuropathy, rash, vomiting, urinary symptoms, global health-related QOL, and interference of symptoms with usual activities" [51]. In attempting to develop a conceptual framework of PROs for CRPC, Eton et al. developed an initial list of CRPC-relevant PROs through a literature review and interviews with patients and clinicians and then compared the resulting list with archived PRO data from a randomized phase III clinical trial of mitoxantrone with prednisone versus prednisone alone. The archived data included both the Prostate Cancer-Quality of Life Instrument (PROSQOLI) and the QOLM-P14. The result is the proposed conceptual framework for CRPC-relevant PROs shown in Fig. 22.1.

Eton et al. also provided a summary of PROs observed at baseline and measured on treatment in clinical trials targeting CRPC patients (Table 22.3). Pain, prostate cancer-specific concerns, and general QOL were most frequent.

Earlier, Yount et al. extracted the most important PROs to monitor for advanced prostate

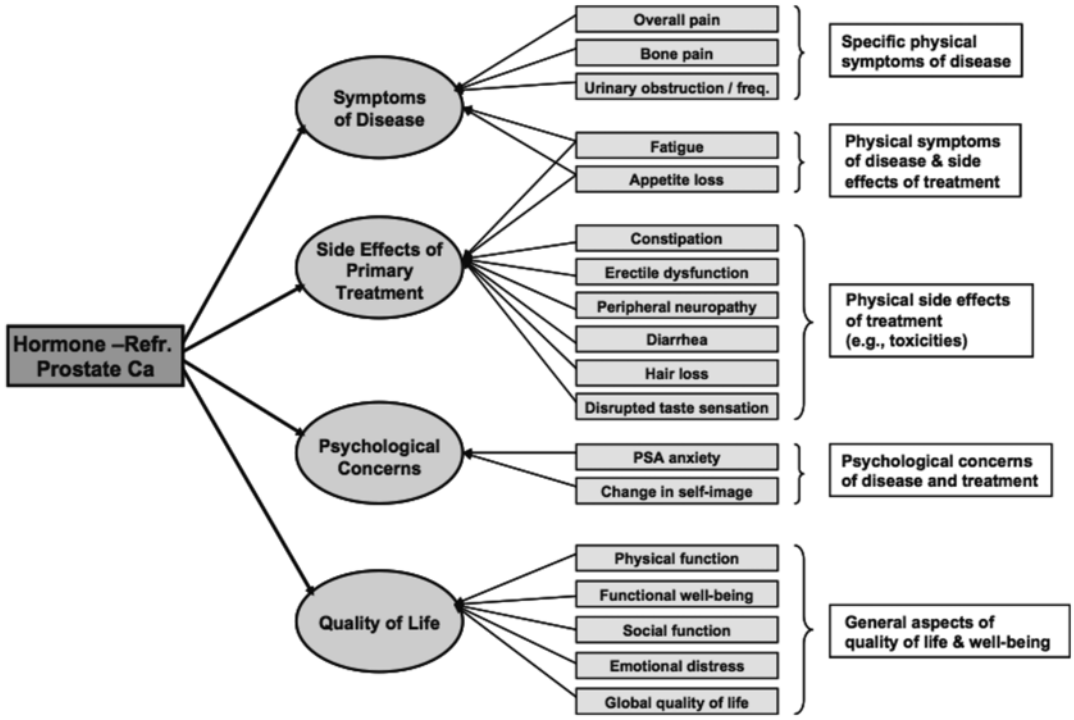

Fig. 22.1 Conceptual Framework for PROs in Castration Resistant Prostate Cancer used with permission from Eton et al. [52] This figure was published in the Journal of the International Society for Pharmacoeconomics and Outcomes Research, 13(5), Eton DT, Shevrin DH, Beaumont J, Victorson D, Cella D, Constructing a conceptual framework of patient reported outcomes for metastatic hormone-refractory prostate cancer, p. 613–23, Copyright Elsevier

Table 22.3 Patient-reported outcomes showing change in HRPC clinical studies (number of times identified per treatment arm)

Concern or domain	Count
Pain	10
Prostate cancer-specific concerns (PCS)[a]	9
Global QOL	8
Emotional function	5
General QOL (total scores of measure—i.e., FACT-P)[b]	5
Fatigue	4
Physical function	4
Nausea/vomiting	3
Analgesic use	2
Functional well-being/role function	2
Depression	1
Appetite	1

[a]Prostate cancer subscale (PCS) of the FACT-P
[b]FACT-P consists of subscales

cancer from a commonly used multi-dimensional prostate-cancer-specific QOL survey, the Functional Assessment of Cancer Therapy-Prostate (FACT-P). Forty-four expert clinicians, each of whom had treated at least 100 patients with advanced prostate cancer over at least 3 years, selected a list of no more than 5 of the "most important symptoms or concerns to monitor when assessing the value of treatment for advanced prostate cancer." The researchers validated the experts' selection in a phase III randomized clinical trial of atrasentan in 288 men with CRPC [53]. Table 22.4 shows the ranked list of PROs that were endorsed by at least 17 % of the experts.

In the following sections we review the character of each of these PROs and the treatments that have proven to be most effective.

Table 22.4 Most important symptoms in CRPC, selected by clinicians

Symptoms/concerns	% Endorsed ("top 5")
Pain	68
Fatigue (lack of energy)	64
Pain limits performance	43
Difficulty urinating	32
Worry condition will get worse	27
Bone pain	25
Weight loss	18
Urinating problems limit activity	18

Common Pain Syndromes and Treatment Strategies for Pain in CRPC Patients

Bone metastases occur in 80 % of men with advanced prostate cancer [54]. The most common site for prostate cancer metastases is bone, especially in the spine, pelvis, humeri and femurs, and less commonly in other areas such as the clivus in the skull. Bone metastases frequently cause substantial pain, pathologic fractures, and can spread and develop into spinal cord compression [55].

Effective control of pain as well as reduction in risk of fracture and cord compression in CRPC depends on prompt recognition of the specific pain syndrome the patient is experiencing. Common pain syndromes in CRPC are shown in Table 22.5.

Implementing the treatment strategies for several of the pain syndromes identified in Table 22.5 requires additional assessments described below.

Focal Bone Pain

External-beam localized radiotherapy generally provides effective control of focal bone pain in patients with castration-refractory disease. The presence of osteolytic lesions or pathologic fractures should be assessed using plain radiographs on areas that appear abnormal on bone scan and are painful, an evaluation that is especially important when weight-bearing sites and extremities are affected.

Epidural Metastasis and Cord Compression

Epidural metastasis is a potentially devastating complication of prostate cancer, and the incidence of epidural cord compression is high in this disease because it frequently metastasizes to the vertebrae and paravertebral region. Epidural spinal cord compression is an emergency complication, and for paraplegia to be prevented, early diagnosis and treatment of epidural metastasis are critical. Managing back pain and preserving bowel and bladder function also require early diagnosis [57, 58].

Middle or upper back pain, especially when it is progressive, is often a warning sign of imminent cord compression [59]. When a CRPC patient reports back pain he should be aggressively evaluated for epidural cord compression. Spinal magnetic resonance imaging (MRI) has almost entirely replaced other methods, such as computed tomographic myelography and conventional myelography, for excluding the possibility of significant epidural disease. Most epidural spinal cord compressions arise from vertebral bodies; soft tissue mass involvement in the paravertebral region is rarely the cause and is usually found only as abnormality on neurologic examination.

Corticosteroids are the most common first therapeutic intervention. High doses of intravenous glucocorticoids are commonly employed, for example, beginning with an intravenous "loading dose" of 10 mg of dexamethasone followed by 4–10 mg every 6 h. Evidence defining the optimal dose has not been reported. Symptoms usually improve quickly with steroids, after which a 2–3 week tapering period is common.

Radiation is the definitive treatment for epidural metastasis and cord compression. With intensity-modulated radiation therapy allowing variable dosing during treatment, spinal and

Table 22.5 Pain syndromes in metastatic castration-resistant prostate cancer (adapted) [56]

Pain syndrome	Initial management	Other therapeutic alternatives
Localized (focal) bone pain	Pharmacologic pain management (narcotics) Localized radiotherapy (special attention to weight-bearing areas, lytic metastasis, and extremities)	Surgical stabilization of pathologic fractures or extensive bone erosions Epidural metastasis and cord compression should be evaluated in patients with focal back pain Radiopharmaceuticals should be considered if local radiation therapy fails
Diffuse bone pain	Pharmacologic pain management (steroids, narcotics) "Multi-spot" or wide-field radiotherapy Radiopharmaceuticals Chemotherapy	Rank ligand inhibitors Bisphosphonates Calcitonin
Epidural metastasis and cord compression	High-dose corticosteroids Radiotherapy Surgical decompression and stabilization should be indicated in high-grade epidural blocks, extensive bone involvement, or recurrence after irradiation	Pharmacologic pain management
Plexopathies caused by direct tumor extension or prior therapy (rare)	Pharmacologic pain management Radiation therapy (if not previously employed) Neurolytic procedures (nerve blocks)	Tricyclics (amitriptyline) Anticonvulsants
Miscellaneous neurogenic causes: – Post-herpetic neuralgia, – Peripheral neuropathies	Careful neurologic evaluation Pharmacologic pain management Discontinuation of neurotoxic drugs: paclitaxel, docetaxel, vinca alkaloids, platinum compounds	Tricyclics (amitriptyline) Anticonvulsants
Other uncommon pain syndromes: – Extensive skull metastasis with cranial nerve involvement, – Extensive painful liver metastasis or pelvic masses	Radiotherapy Pharmacologic pain management Corticosteroids (cranial nerve involvement)	Chemotherapy Intrathecal chemotherapy may ameliorate symptoms of meningeal involvement; regional infusions may be considered

paraspinal tissue can be spared and higher doses of radiation can be delivered to the target [60], enabling delivery of therapy within close proximity to the spinal cord with minimal toxicity. For patients who present with evidence of progressive signs and symptoms during radiotherapy, develop or present with unstable pathologic fractures, or have recurrence after radiotherapy, circumferential spinal cord decompression surgery may also be considered [61]. In considering surgery, the overall prognosis of CRPC should be taken into account. Chemotherapy is generally not used to treat epidural cord compressions (Table 22.6).

Treatment Strategies for Other PROs Frequently Encountered with CRPC Patients

Anxiety and Depression

As shown in Table 22.2, most PROMs measure anxiety and depression, and prostate cancer-specific PROs such as FACT-P also measure patient concern about disease progression reflecting the common concerns reported by CRPC patients. Patients reporting anxiety and/or depression may be encouraged to exercise, or an antidepressant may be prescribed. In addition, they

Table 22.6 Comparison of pain relief from alternative treatment strategies including hormonal treatment, chemotherapy, radiation, bone-targeting drugs, and experimental targeted agents

Category	Trial	Pain comparison
Second line hormonal therapy	Abiraterone acetate versus placebo [62]	Pain palliation: 45 % versus 28.8 % ($p=0.0005$); Speed of palliation 5.6 versus 13.7 months ($p=0.0018$)
	Abiraterone acetate versus placebo [29]	Median time to pain progression 26.7 versus 18.4 months Median time to progression of pain interference with daily activities: 10.3 versus 7.4 months ($p=0.005$)
	Enzalutamide versus placebo [63]	Pain severity −7.5 % versus +23 % ($p<0.001$)
Chemotherapy	Docetaxel versus mitoxantrone [64]	No significant difference
	Cabazitaxel versus mitoxantrone [43]	No significant difference: 9.2 % versus 7.7 % ($p=0.63$)
Radiation	Sm-153 versus placebo [65]	72 % pain relief ($p<0.034$)
	Sr-89 [66]	Mean complete pain response 32 %, mean partial pain response 44 %
	Ra 223 versus placebo [67]	NE
Bone-targeting drugs	Zoledronic acid versus placebo [68]	Mean pain score: 0.43 versus 0.88 ($p=0.026$)
	Denosumab versus zoledronic acid [69]	NE
Experimental targeted agent	Cabozantinib [70]	67 % of patients had pain improvement

NE = not evaluated

may be referred to a social worker or support group for comfort and coping skills. For severe cases, patients may be referred to psychiatrists.

Fatigue

Fatigue is measured by nearly all PROMs because many treatments for cancer, including radiation therapy, chemotherapy, and molecularly targeted agents, are accompanied by patient-reported fatigue that may be disabling. Targeting the etiology of the fatigue may inform treatment choices. For example, if the fatigue arose from anemia caused by chemotherapy, blood transfusions may be prescribed. More generally, cancer patients are advised to rest when tired. Inactivity may lead to muscle wasting, however, and patients are often encouraged to participate in regular physical exercise programs which often lead to reduced fatigue and greater physical capacity [71], although published trials are limited to patients recovering from curative chemotherapy rather than CRPC patients. Pharmacologic management with psychostimulants such as methylphenidate and dextroamphetamine has minimal effect on cancer-related fatigue, even though they work in other diseases, and the effect is restricted to patients with severe fatigue (greater than 8 on a scale of 0–10) [72, 73]. Wisconsin ginseng at 2,000 mg a day was effective in reducing cancer-related fatigue in a randomized trial of 364 participants, with no toxicity. However, fewer than 10 patients had prostate cancer [74]. Steroids such as methylprednisolone, dexamethasone, or prednisone can also reduce fatigue as well as pain and nausea [75, 76].

Urination Problems

Urinary problems are not measured by most general PROMS, but prostate cancer PROMs

like FACT-P and EPIC do measure urinary distress. Because urinary dysfunction may have multiple causes, including benign prostatic hypertrophy causing bladder outlet obstruction, overactive bladder, or obstruction due to local recurrence of the prostate cancer or cystitis from external-beam radiation or brachytherapy, or infection, patients are often referred to their urologists for diagnosis and treatment. However, obstruction and/or hematuria caused by local recurrence of the prostate cancer is often treated with chemotherapy.

Weight Loss

Although weight loss is not measured by general PROMs, weight loss is included in FACT-P. Standard therapies are appetite stimulants such as megestrol acetate and glucocorticoids; dronabinol (Marinol) has less effect than megestrol acetate [77].

The Future of PROs and PROMs in CRPC

PROs are critical factors in treatment selection for CRPC patients. Progressive back pain, in particular, can be an indicator of imminent devastating cord compression and should trigger immediate evaluation to rule out epidural cord compression. Two key advances in PROs may lead to better communication between patients and their oncologists and more rapid response to early indicators of catastrophic complications. First, agreement needs to be reached on consistent reliable measures of PROs including pain. Second, PROs need to be more quickly and reliably reported, using advances in electronic solutions, so they can be reported on an ongoing basis and evaluated in a timely manner to enable appropriate care to be given. At the same time, PROs should be integrated into electronic health records and clinical trials to enable comparative effectiveness research [44].

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Evidence-Based Therapeutic Approaches for mCRPC Patients: Rational Sequence of Standard Treatment Options and Design for Future Drug Development

Jacob A. Martin and William K. Oh

Introduction

Over the last 3 years, at least five new anticancer therapies have been approved for the treatment of metastatic castration-resistant prostate cancer (mCRPC): cabazitaxel, sipuleucel-T, abiraterone, enzalutamide, and radium-223. These treatments—along with docetaxel, which was approved in 2004—represent the current list of agents that have demonstrated a survival benefit in patients with mCRPC. Each is capable of extending survival for individuals with metastatic prostate cancer that is resistant to primary androgen deprivation therapy; however, none of these treatments has been shown to be curative. Therefore, the state of the art treatment for mCRPC remains associated with progression after multiple different therapeutic regimens. Appropriately sequencing and possibly combining these treatment regimens will be crucial for maximizing survival and minimizing adverse events.

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Unfortunately, there is currently little prospective evidence for when to use each available therapy. Given the rapid pace of drug discovery, each of the recent approvals came as a result of comparison either to placebo, or—in the case of cytotoxic chemotherapy—randomization against mitoxantrone, a chemotherapy drug that does not improve survival. Clinical trials of new drugs in sequence logically come after trials that establish their initial safety and efficacy. Therefore, it is expected that studies on drug sequencing and combinations will lag behind their single-drug counterparts. Moreover, studies of multiple drugs in sequence are clearly more difficult to complete than trials of single agents tested against placebo or existing treatments. Finally, preclinical models of specific sequential therapies are rarely performed and often do not demonstrate predictive value. For instance, it is extremely difficult to model a course of chemotherapy followed by immunotherapy in current animal models.

Existing treatments for mCRPC can be classified into one of four broad groups based on their mechanisms of action: (1) androgen signaling pathway inhibitors (2) immunotherapy (3) cytotoxic chemotherapy, and (4) bone-directed therapies. Though each has been shown to improve survival, all of these approaches eventually become ineffective as the tumor cell population develops resistance. Since treatment itself may foster tumor resistance, this can consequently impact the value of sequential therapies. It is critical to understand these changes in tumor sensitivity in order to know which therapies (if any) should precede others.

F. Saad and M.A. Eisenberger (eds.), *Management of Castration Resistant Prostate Cancer*, Current Clinical Urology, DOI 10.1007/978-1-4939-1176-9_23,

Additionally, we do not yet understand which drugs should be given together to create additive or synergistic combinations. Effective combinations may be found within classes, such as two anti-androgenic agents, or between classes, such as a bone-targeted therapy concurrent with an androgen pathway inhibitor. In either case, combinations seek to block or prevent paths of drug resistance and to create more effective ways to induce tumor cell death.

In studying these new therapies, it has also become clear that prostate cancer can be very heterogeneous in its initial presentation, manner of progression, and response to therapy. For instance, there are clinical trials in which the treated group did not benefit enough to improve overall survival, but in which some individuals have been shown to have dramatic responses. It is imperative to understand what distinguishes good and poor responders for a particular therapy in order to limit unnecessary toxicity and also to achieve the best response to a given therapy, even if only in a subset of patients with mCRPC. Biomarkers are emerging that may help predict survival [1]. The next step will be to uncover biomarkers that predict the most effective and safe treatment response and thus sequence of therapy.

Recent advances in drug development have improved the outlook for patients with mCRPC, though the disease remains fatal. Over the next few years, trials of drugs in sequence and in combination will be conducted to contextualize new therapies within the array of treatment options. Additionally, we must develop better means of understanding individual tumors and selecting the best responders to specific treatments. In this chapter, we will review the available data to help tailor drug selection and timing for men with mCRPC.

Optimal Timing of Immunotherapy

Sipuleucel-T is the first immune therapy approved for treatment of mCRPC or indeed any cancer. In the IMPACT study, sipuleucel-T demonstrated a 4.1 month improvement in overall survival in men with asymptomatic or minimally symptomatic mCRPC [2, 3]. This population was selected because the immune response is thought to act over months or years to inhibit tumor progression. Early use of sipuleucel-T in mCRPC could thus hopefully provide the most benefit. In the IMPACT study, sipuleucel-T paradoxically did not show a significant improvement in time to progression or clinical response compared with placebo. In fact, sipuleucel-T rarely induces significant PSA declines and does not appear to palliate symptoms of metastatic disease. Therefore, patients with symptomatic disease usually require treatments such as chemotherapy or androgen pathway drugs that can more effectively improve cancer-related symptoms.

The optimal timing for the initiation of sipuleucel-T in patients with mCRPC remains uncertain. Schellhammer et al. recently reported a post-hoc analysis of the IMPACT trial in which patients were grouped into quartiles based on their baseline PSA level on study entry [4]. This analysis demonstrated that both the absolute survival difference and the hazard ratios for survival were significantly improved in the lower quartiles of PSA. Since baseline PSA represents a measure of disease burden, this study suggests that the maximal benefit of sipuleucel-T may be when the PSA and thus overall disease burden with mCRPC is lowest.

Giving sipuleucel-T earlier in asymptomatic mCRPC may also have other advantages. Sipuleucel-T has a favorable side-effect profile, with just 6.8 % of patients in the IMPACT trial reporting adverse events of grade 3 or more, compared to 1.8 % in the placebo group [3]. Side effects were most commonly flu-like symptoms, such as fever, chills, and rigors. Sipuleucel-T's toxicity compares favorably to the other treatments for mCRPC since it may preserve a patient's quality of life compared with treatments like chemotherapy, which have been associated with more adverse effects.

Another important factor to consider when sequencing sipuleucel-T and other immune therapies is whether the patient is concurrently being treated with drugs that could suppress the immune system. Since sipuleucel-T works by targeting

the immune system against the tumor-specific antigen, prostatic acid phosphatase, it requires an intact immune system to be effective. Abiraterone acetate is given with prednisone, which could have a suppressive effect on immunotherapy. Chemotherapy is also often accompanied by corticosteroids, both of which could be similarly problematic for ongoing immune therapy.

The interaction between chemotherapy and sipuleucel-T is poorly understood. No randomized trials have studied the two therapies concurrently or in a specific sequence. On one hand, it is understood that chemotherapy induces apoptotic cell death—a form of destruction that is not regarded as immunogenic. Additionally, chemotherapy is known to be immunosuppressive. However, a recent study suggests that tumor cells being destroyed by chemotherapy may have important interactions with the immune system that could enhance response to immunotherapy [5].

Further studies are required to definitively place sipuleucel-T in sequence with other available therapies for mCRPC. However, our current knowledge allows us to safely give sipuleucel-T to patients with metastases who have progressed on first-line hormone therapies yet do not have symptoms that warrant treatment with drugs which induce immediate anticancer responses, such as chemotherapy or androgen pathway inhibitors. In this population, patients receiving sipuleucel-T have an overall survival benefit compared to patients receiving placebo, with few side effects—an ideal cost-benefit balance for the asymptomatic or minimally symptomatic mCRPC patient.

When Should Androgen Pathway Therapies Be Used Relative to Chemotherapy?

While sipuleucel-T has not been shown to palliate symptomatic mCRPC, androgen pathway drugs, cytotoxic chemotherapy, and radium-223 have all been associated with both a survival benefit and the ability to induce symptomatic responses in patients with mCRPC. Given the recent approval of radium-223 in May 2013, little is yet known about exactly when it should be used in practice. However, we now have several years of experience and more data on the relationship between androgen pathway drugs and chemotherapy. A crucial dilemma lies in the sequencing of these two types of treatments. In 2004, docetaxel was the first agent to show an improvement in survival for patients with mCRPC in phase III clinical trials [6, 7]. Over the past 3 years, abiraterone was approved for use after chemotherapy and then in chemo-naïve patients [8, 9]. Similarly, enzalutamide was approved for use after chemotherapy [10], with an ongoing phase III trial in chemo-naïve patients recently reported to have met its primary endpoint (NCT01212991). While we do not have a definitive sequence of chemotherapy and androgen pathway agents unless a randomized trial directly studies this question, existing data provide some clues as to a rational sequence of these two key classes of drugs.

Two registrational phase III trials compared abiraterone plus prednisone to prednisone alone. COU-AA-301 enrolled patients who had previously received docetaxel chemotherapy [8] while COU-AA-302 enrolled patients naïve to chemotherapy [9]. While it is not possible to make definitive conclusions by comparing phase III trials, there is a suggestion from these studies that there may be an advantage to giving abiraterone before chemotherapy. While hazard ratios for survival were similar pre- and post-chemotherapy, both the absolute benefit and hazard ratio for radiographic progression-free survival (rPFS) were significantly better in the pre-chemotherapy trial. rPFS was improved by 8 months (HR 0.53) in patients given abiraterone before chemo and 2 months (HR 0.65) in patients give abiraterone after chemotherapy [8, 9, 11]. While not definitive, there is a suggestion that the maximal benefit from abiraterone may be seen if it is used before chemotherapy.

Additionally, abiraterone may be a better choice earlier in the disease course based on its more favorable toxicity profile. Approximately a third of chemo-naïve patients on abiraterone plus prednisone reported serious adverse events [9]. Peripheral edema, arthralgia, and fatigue were among the most common adverse events in abiraterone treated patients. Docetaxel, on the other

hand, is associated with an increased incidence of more serious side effects. Twenty-six percent of patients treated with docetaxel and prednisone every 3 weeks experienced serious side effects such as neutropenia, diarrhea, dyspnea, sensory neuropathy, peripheral edema, and fatigue. Abiraterone plus prednisone increased the median time to initiation of cytotoxic chemotherapy by 50 % ($p<0.001$) [9]. Thus, using abiraterone therapy prior to chemotherapy could be a means of maximizing progression-free survival in mCRPC, while delaying the use of potentially more toxic chemotherapeutic agents.

Survival analysis of the two phase III abiraterone trials demonstrates several interesting findings. After docetaxel chemotherapy, the hazard ratio for overall survival for the abiraterone arm was 0.75 after a median follow-up of 20.2 months [11]. In the study of chemo-naïve patients receiving abiraterone, the hazard ratio for overall survival was *also* 0.75 [9]. This identical hazard ratio for the two groups suggests that chemotherapy may not have influenced resistance rates to subsequent androgen pathway therapies. Obviously, there are important limitations to this type of comparison, including the fact that the two studies enrolled groups of patients that were substantially different. Patients treated with abiraterone after completing chemotherapy were clearly more ill and advanced in their disease state. Patients in the pre-chemotherapy study survived substantially longer regardless of treatment with abiraterone. In addition, 46 % of the chemotherapy-naïve patients went on to receive docetaxel or cabazitaxel after completing abiraterone [9], which likely conferred an additional survival benefit. Based on existing data, one cannot conclude with certainty that abiraterone would have a differential effect on overall survival if used before or after docetaxel chemotherapy.

Interestingly, preclinical studies do suggest that taxane chemotherapy may interact with androgen pathway therapies, possibly creating cross-resistance [12]. Taxane chemotherapy interferes with microtubule assembly, specifically by stabilizing the mitotic spindles that are essential for segregating genetic material during cell division. Recently, however, it has been shown in vitro that docetaxel has a down-regulatory effect on expression of both AR and PSA, and conversely that overexpression of AR can mitigate the cytotoxic effects of docetaxel chemotherapy [13]. Additional studies have shown that taxanes cause accumulation of the AR-suppressive factor, FOXO1, in the nucleus and may inhibit AR translocation to the nucleus for signaling [14–16]. There is some clinical data to support these findings. In a retrospective study, patients who did not respond to abiraterone were subsequently found to be poor responders to docetaxel [17]. Of 35 patients treated with abiraterone, 8 failed to achieve any decline in PSA. All of these individuals were also deemed resistant to docetaxel. Thus, while hazard ratios were similar for progression on abiraterone before and after chemotherapy, there may be an unseen interaction between the two therapies that will become clearer in future trials.

While the existing evidence suggests that abiraterone may confer the greatest benefit in rPFS when given prior to chemotherapy, the overall survival benefit as measured by hazard ratios is the same in trials of abiraterone prior to and after docetaxel chemotherapy. Moreover, preclinical data suggests that chemotherapy and abiraterone may share pathways of cross-resistance. In the absence of a randomized trial to test this question, it remains impossible to definitively state that abiraterone should come before or after docetaxel chemotherapy.

Another important androgen pathway therapy to consider is enzalutamide—the other major approved drug in this class. The AFFIRM phase III trial found that enzalutamide was associated with a significant survival benefit after chemotherapy. The PREVAIL (NCT01212991) phase III study of enzalutamide in chemo-naïve patients has recently been reported to also confer an overall and progression-free survival benefit, in preliminary reports. Once these trials are fully reported, we will be able to more effectively use androgen pathway drugs and chemotherapy in sequence to improve outcomes in mCRPC.

Sequencing Newer Androgen Pathway Inhibitor Therapies

Since 2012, both abiraterone and enzalutamide have been approved as second-line androgen pathway inhibitor therapies for mCRPC. Clinical trials have not yet been conducted to determine their optimal sequence relative to one another or, as discussed above, relative to other available treatments such as chemotherapy. However, retrospective studies may provide some clues for optimizing their use in the management of prostate cancer. For instance, some patients from each registrational randomized trial went on to receive the alternate androgen pathway inhibitor therapy (e.g., abiraterone after participating in a phase III trial of enzalutamide). Overall, the aggregate response appears to be modest. However, some patients respond exceptionally well after crossing over to the alternative androgen pathway agent. This recapitulates a familiar story in cancer therapy, both regarding the fact that tumor biology is exceedingly diverse and that some pathways can be repeatedly exploited therapeutically in some patients. Further studies must be done to understand the mechanisms of resistance and uncover relevant predictors of response.

The first sequential androgen pathway inhibitor studies reported responses in patients receiving abiraterone after progression on enzalutamide post-chemotherapy. Two groups retrospectively studied participants in the AFFIRM phase III study of enzalutamide after they progressed and were switched to abiraterone [18, 19]. Both studies used PSA declines as the primary endpoint. Overall, clinical responses were modest. A minority of participants, however, benefited greatly from abiraterone therapy. Ten of 68 patients (15 %) had a PSA reduction of at least 30 % and 4 (6 %) had a PSA decline of at least 50 %. There was no apparent correlation between the initial response to enzalutamide and subsequent response to abiraterone. Among the four patients with ≥50 % PSA reduction on abiraterone, two previously experienced ≥50 % PSA reductions on enzalutamide, one had a ≤50 % PSA reduction, and one had an 8 % PSA increase.

A similar retrospective study was conducted to evaluate patients receiving the opposite sequence: enzalutamide following progression on abiraterone in the post-chemotherapy setting [20]. Again, some patients responded to a second-line androgen pathway inhibitor therapy. Of the 35 patients, 10 (29 %) had a decline in PSA greater than 50 %. These numbers appear moderately better than the retrospective abiraterone studies. However, they also indicate that most patients build cross-resistance to androgen pathway inhibitors, with the majority of patients either not responding or having a modest response to enzalutamide. Overlapping mechanisms of resistance between abiraterone and enzalutamide likely explain these findings.

The results of these retrospective studies suggest that some patients continue to harbor androgen sensitive tumors even after failing chemotherapy and first-line androgen pathway inhibitor therapy; there is a need to better identify such "androgen pathway addicted" tumors. In general, metastatic treated tumors are believed to trend toward dedifferentiation [21], but some cancers retain a differentiated phenotype, at least to the extent that they continue to respond to androgen pathway drugs.

In summary, these studies do not strongly support the routine use of second-line androgen pathway inhibitor therapy following failure of first-line androgen pathway drugs. Preclinically, both enzalutamide and abiraterone cause upregulation of constitutively active androgen receptor splice variants in vitro [22, 23]. Crosstalk between the AR pathway and PI3K may recapitulate the signaling needed for prostate tumor growth when either pathway is inhibited independently [24]. Resistant tumors may need the addition of drugs that block these alternative pathways.

Who Is the Appropriate Patient for Radium-223 and When Should It Be Used?

Radium-223 is a bone-directed therapy that has demonstrated a survival benefit in CRPC patients with bone metastases. In addition, it has been associated with a favorable side-effect profile.

Radium-223 is able to target areas of high bone turnover and release powerful but short-range alpha radiation. Thus, it is able to generate minimal side effects while having a potentially strong anti-tumor effect in bone. In the ALSYMPCA study, radium-223 improved overall survival by 3.6 months (HR 0.70; $p<0.01$) [25]. It also significantly increased time to the first symptomatic skeletal event (HR 0.70) and time to PSA increase (HR 0.64). Adverse events were primarily mild and occurred a lower rate in the radium-223 group compared to controls.

Existing research supports the use of radium-223 in patients with symptomatic bone metastases and CRPC. In addition to improvements in survival and symptoms, the generally low risk of adverse events and overall survival benefit makes radium-223 suitable for symptomatic CRPC patients with metastatic disease. Nonetheless, important questions must still be answered in order to place radium-223 in sequence with other therapies. First, patients with visceral metastases were excluded from the ALSYMPCA trial. Therefore, it is unclear whether such patients with bone and visceral metastases would benefit from radium-223. Second, patients were excluded who were considered "unfit" for docetaxel or who had declined to receive it. How to define a population "unfit" for docetaxel is controversial, especially considering that many of these patients subsequently did receive docetaxel after radium-223. Finally, radium-223 must be studied further in sequence and combination with other agents. The ALSYMPCA trial allowed other treatments to be used concurrently, including external beam radiotherapy, androgen pathway drugs, and bisphosphonates. However, cytotoxic chemotherapy was not allowed concurrently. Even though other approved drugs do not act on the same pathways as radium-223, little is known currently about what sequence will provide the best clinical outcome.

A Reasonable Approach

In the absence of curative treatments for mCRPC and conclusive data on optimal sequencing and/or combination therapies, one reasonable strategy is to design personalized regimens based on factors including patient age and performance status, patient preference, drug side-effect profiles, sites of cancer metastasis, history of prior therapies and response, and cost-related issues. Since the hazard ratios for each of the phase III trials were similar, it is difficult to say that one drug is clearly superior to another from an efficacy standpoint. Also, for each reported clinical trial, we understand the adverse events associated with each therapy and so starting with less toxic treatments may delay progression while minimizing impact on the patient's quality of life.

Our approach has been to first confirm the presence of CRPC by documenting a castrate level of serum testosterone (<50 ng/dL), continuing lifelong LHRH agonist or antagonist therapy, and to add therapies in sequence as follows:

1. Earlier therapies (asymptomatic-minimally symptomatic)
   a. Anti-androgens such as bicalutamide
   b. Sipuleucel-T
   c. Abiraterone acetate/prednisone
   d. Enzalutamide
2. Later therapies (symptomatic)
   a. Radium-223 (bone metastasis)
   b. Docetaxel chemotherapy
   c. Abiraterone or enzalutamide (if not used already)
   d. Cabazitaxel (after docetaxel)
3. Other considerations
   a. Bone supportive care (zoledronic acid or denosumab)
   b. Platinum chemotherapy (small cell/neuroendocrine differentiation)
   c. Palliative bone radiation
   d. Other chemotherapy (rarely mitoxantrone, vinorelbine, cyclophosphamide)
   e. Other androgen pathway drugs (bicalutamide, nilutamide, ketoconazole)
   f. Clinical trials whenever possible

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F. Saad and M.A. Eisenberger (eds.), *Management of Castration Resistant Prostate Cancer*, Current Clinical Urology, DOI 10.1007/978-1-4939-1176-9, © Springer Science+Business Media New York 2014