Prostate Cancer

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eXon Publications

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eXon
PUBLICATIONS

Prostate Cancer
ISBN: 978-0-6450017-5-4
DOI: https://doi.org/10.36255/exonpublications.prostatecancer.2021

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Published by
Exon Publications, Brisbane, Australia

First Published in May 2021
Printed in Australia

CONTENTS

https://doi.org/10.36255/exonpublications.prostatecancer.2021.frontmatter

FOREWORD

Prostate cancer is a life-altering diagnosis, not just for patients, but for their loved ones as well. Once, prostate cancer was most frequently diagnosed in men in their 60s and 70s; with increasing use of imaging modalities and PSA testing, the incidence is on the rise, and we are now seeing men in their 40s with the disease. This poses a challenge for clinical management depending on the stage of the disease—from watchful waiting or active surveillance to aggressive treatment with the risk of therapeutic nihilism. While localized prostate cancer diagnosed at early stages is curable in most cases, the prognosis for metastatic disease is poor. Most patients with advanced disease will develop resistance to therapy and we need to understand more about the disease and develop efficacious therapeutic strategies for prostate cancer.

This book enhances our understanding of this disease in many ways: identifying who we need to diagnose, those most at risk based on their genetic profile, and also where diagnosis and treatment may not be in an individual's best interests. It looks into the future where the diagnostic pathway merges with the treatment with theranostics and reflects on lessons we can learn from the past. This book is a comprehensive and current account, covering the whole sphere of prostate cancer.

The editors, Simon Bott and Keng Ng, are to be congratulated for amassing such stellar contributors who together have produced a book that is easy to read but also highly informative. I enjoyed reading each chapter and I would encourage all those involved in the world of prostate cancer to do the same.

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Doi: https://doi.org/10.36255/exonpublications.prostatecancer.foreword.2021

PREFACE

Prostate cancer has become one of the world's greatest healthcare challenges. Ageing populations mean the number of men diagnosed with this disease increases year on year. This has implications not only to the patient and their loved ones, but also clinical services, healthcare budget holders and to wider society. Despite being one of the most common cancer in Western men, its natural history, prognosis, and treatment are poorly understood. This book brings together a select faculty of experts to present a comprehensive view of the current state and future perspectives of prostate cancer.

There are ten chapters in the book—the first four cover our present knowledge and understanding of the disease, the following three explore new advancements and treatments, particularly looking at overcoming resistance to therapy, and the remaining three chapters focus on specific molecules with the potential to become drug targets.

Chapter 1 provides a comprehensive review of the global trends in epidemiology, geographical variations, and incidence and mortality of prostate cancer. With numerous modifiable and unmodifiable risk factors, the etiology of prostate cancer is comprehensively covered. Chapter 2 summarizes the current knowledge on the various etiological factors of prostate cancer. Our understanding of the mechanisms that cause the disease requires further expansion if we are to advance our ability to diagnose aggressive tumors and develop more effective therapies. Chapter 3 provides a snapshot of pathogenesis of prostate cancer. The diagnostic tools for prostate cancer have undergone significant advancements in recent years to improve the accuracy of prostate cancer detection and avoid overdiagnosis and subsequent overtreatment. Chapter 4 introduces the reader to the various biochemical, genetic, imaging, and histological modalities that enable the accurate diagnosis and staging of the disease. Together, these four chapters serve as a succinct reference tool not only for clinicians or researchers, but also for the layperson who wish to have a basic understanding of the disease.

Chapters 5, 6 and 7 give a glimpse into future prospects for the treatment of prostate cancer. Androgen-deprivation therapy is the standard of care for metastatic prostate cancer, but patients inevitably develop resistance to treatment; more efficient treatments are therefore necessary. One such possibility is the combination of androgen deprivation with other forms of treatments such as immunotherapy, radiation, or small molecule inhibitors. Chapter 5 summarizes these promising combination treatment strategies and provides a comprehensive list of clinical trials on the topic. Chapter 6 introduces the reader to the exciting field of theranostics, which involves imaging a particular molecular target with a diagnostic radioisotope and then substituting it with a therapeutic isotope to treat patients who demonstrate sufficient target expression on diagnostic imagery. This is topical, given the FDA approval in December 2020 of PSMA-targeted PET imaging for men with prostate cancer. Improvements in systemic therapies need to be developed and applied in a timely, strategic manner to improve the care of those at the most extreme risk of therapy failure with traditional therapy.

Chapter 7 reviews the status of therapy for standard and high-risk patients, and strategies for translational science for patients at risk of compromised outcome and treatment failure.

Chapters 8, 9, and 10 will be of particular interest to basic scientists, as they discuss the therapeutic potential of three molecules. Chapter 8 challenges researchers to rethink the role of p53, often dubbed the 'guardian' of the genome, in prostate cancer. Originally thought to be involved in metastatic disease, emerging data show that p53 is also dysregulated in primary tumors. Chapters 9 and 10, summarize the current evidence we have on the role of MUCIN 1 and STEAP proteins, respectively, and make a case for their therapeutic potential for the treatment of prostate cancer.

We thank the authors for their contribution, diligence, and dedication for making this project possible. This book is aimed primarily at clinicians and scientists, but many areas will also be of interest to the layperson. We all have much to learn about prostate cancer. We hope this book enhances the reader's knowledge in an informative and enjoyable way.

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May 2021
Doi: https://doi.org/10.36255/exonpublications.prostatecancer.preface.2021

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Doi: https://doi.org/10.36255/exonpublications.prostatecancer.2021.contributors

1

The Epidemiology of Prostate Cancer

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Doi: https://doi.org/10.36255/exonpublications.prostatecancer.epidemiology.2021

Abstract: Prostate cancer is the third most common diagnosed malignancy. It is a heterogeneous disease with incidence rates that vary substantially across the world, from 6.3 to 83.4 per 100,000 people. Age-standardized incidence rates are the highest in Northern Europe and lowest in South Central Asia. Men of African origin are more prone to the disease compared with other ethnicities. Mortality rates differ significantly from incidence rates, with the highest figures in the Caribbean, Sub-Saharan Africa and Micronesia/Polynesia. This chapter provides an overview of the global trends in epidemiology of prostate cancer. Incidence and mortality rates in the Americas, Africa, Europe, Asia, and Oceania are presented.

Keywords: epidemiology; incidence; mortality; prostate cancer; prostate-specific antigen

In: *Prostate Cancer*. Bott SRJ, Ng KL (Editors). Exon Publications, Brisbane, Australia.
ISBN: 978-0-6450017-5-4; Doi: https://doi.org/10.36255/exonpublications.prostatecancer.2021

INTRODUCTION

In 2020, according to the World Health Organisation (WHO), prostate cancer is the third most common diagnosed malignancy. With 1,414,259 cases (7.3% of the total), prostate cancer is preceded only by lung and colorectal cancer with 2,206,771 and 1,148,515 cases respectively (11.4 and 10.0%) (1). It is the most commonly diagnosed cancer in over 50% of countries in the world (112 of 185) and its incidence varies substantially between countries with a high Human Development Index (HDI) and those with a low HDI, 37.5 vs 11.3 per 100,000 people, respectively. Mortality rates are less variable (8.1 vs 5.9 per 100,000 people). Prostate cancer is a heterogeneous disease with incidence rates that vary substantially across the world from 6.3 to 83.4 per 100,000 people. The regions with highest figures are Northern and Western Europe, the Caribbean, Australia/New Zealand, North America and Southern Africa (Figure 1). The lowest are found in Asia and North Africa. However, cases are increasing in Asian countries such as Japan and Singapore where, historically, this cancer had a low incidence rate and prostate-specific antigen (PSA) testing was minimal (2). Mortality rates differ significantly from incidence rates, with the highest figures in the Caribbean (75.8 per 100,000 people), Sub-Saharan Africa (22.0 per 100,000 people) and Micronesia/Polynesia (18.8 per 100,000 people) (1). This chapter provides an overview of the global incidence and mortality of prostate cancer.

PROSTATE CANCER IN THE USA

The most recent statistics published on prostate cancer by the National Center for Health Statistics (NCHS), USA, includes data from 1930–2017. This is due to a delay between data collection and analysis of about 3–4 years. Based on this assumption, the AMC (American Cancer Society) made the following prediction for 2020: (i) 606,520 total cancer deaths, of which, 33,330 (5.5% of total) were expected to be from prostate cancer; and (ii) 1,806,590 new cases of cancer, of which, 191,930 are prostate cancers (10.6%). Prostate cancer is the third most common tumor after lung and colon cancer, and the second most deadly after lung cancer. According to the SEER model (Surveillance, Epidemiology and End Results), the lifelong probability of an American developing an invasive prostate cancer is 11.6 (1 in 9) (3).

The incidence of prostate cancer is strongly correlated to the changes in medical practice and PSA monitoring programs. The early 1990s witnessed a sharp increase in cases. This was due to the widespread introduction of PSA monitoring (formally approved by FDA in 1986), which dramatically increased the detection of asymptomatic disease (4). Those figures then declined quite suddenly between 2007 and 2014 and stabilized around 2016. Prostate cancer incidence rates constantly declined by 6.5% per year from 2007 for all races combined. In contrast, for advanced stage disease, there was an inflection point involving all racial groups and ages (5). The explanation could partially rely on the fact that in 2012, the US Preventing Services Task Force recommendations were changed against routine PSA testing (Grade D) due to concerns about prostate cancer overdiagnosis and overtreatment (6, 7). Thus, after years of ‘excitement’, clinicians started testing

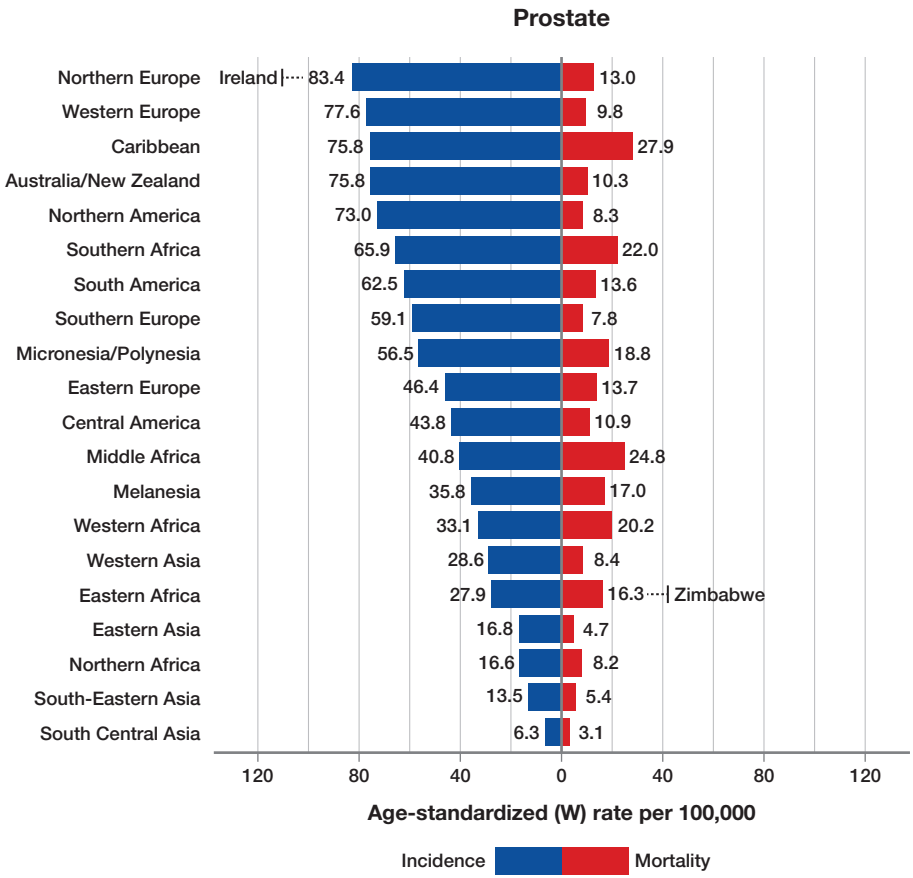


Figure 1. Region-specific incidence and mortality. Age-standardized rates for prostate cancer in 2020. Rates are shown in descending order of the world (W). Age-standardized incidence rate, and the highest national age-standardized rates for incidence and mortality are superimposed. Source: GLOBOCAN 2020. https://gco.iarc.fr [Accessed on 06 April 2021].

less and less patients. However, the increase in advanced stage diagnosis cannot completely be explained by the change in PSA screening protocols (Figure 2).

As explained by Negoita in his paper in 2018, those figures could also be partially explained by improved staging workup or better stage documentation (5). In 2018, the same US Task force revised their recommendation to "informed decision making" for men between 55 and 69 years (Grade C). This was due to updated evidence that showed "a small potential benefit" of reduced prostate mortality in some men (8, 9). An important remark must be made regarding prostate cancer incidence and ethnicity. Much research has shown that the incidence of prostate cancer is greatest in African American men (1). This demographic is generally more likely to develop prostate cancer at any age and develop the cancer earlier in life than men of any other racial or ethnic group (Figure 3). Studies have been done to compare incidence rates of the same ethnic group between different

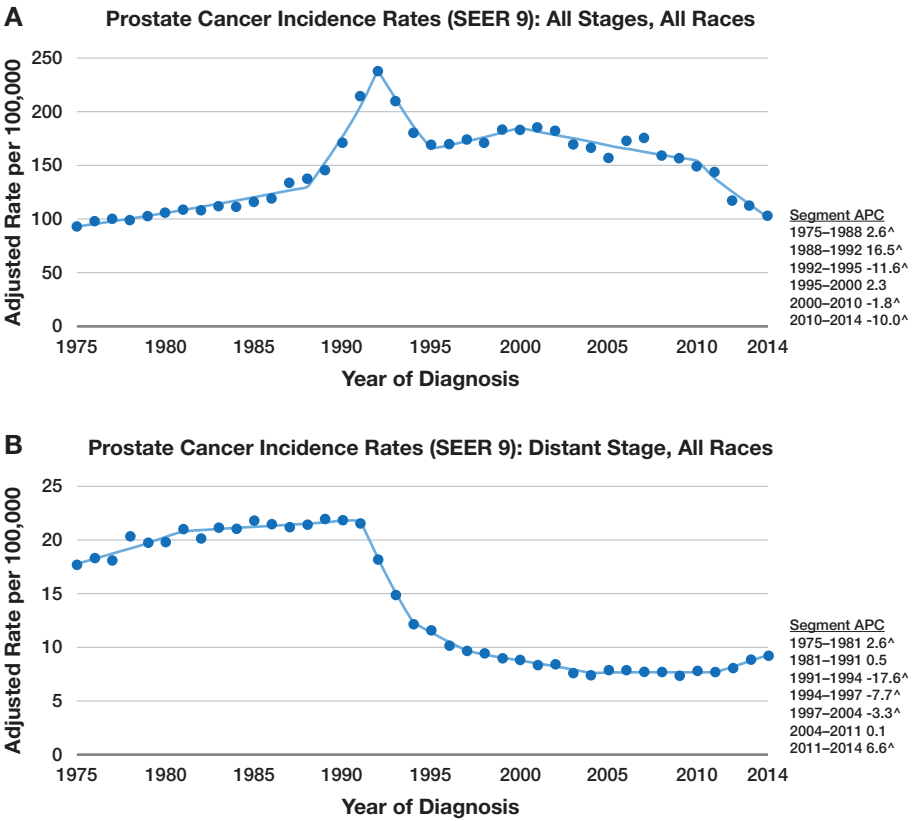
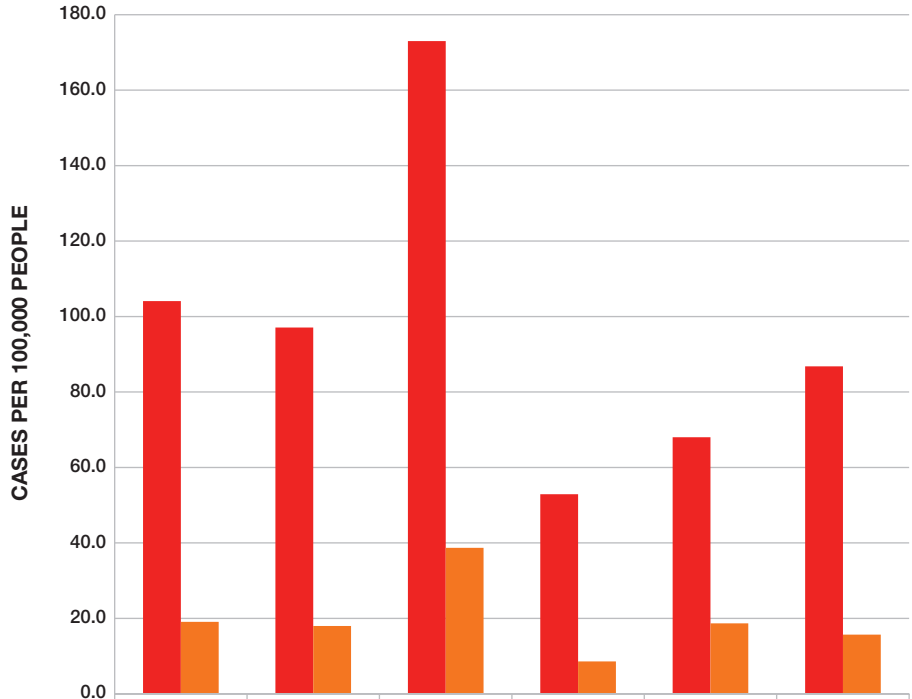


Figure 2. Prostate cancer incidence rates (SEER 9). A. All stages and all races. **B.** Distant stage and all races. Rates are per 100,000 persons and have been age-adjusted to the 2000 US standard population and delay-adjusted for age and stage at diagnosis. APC (annual percent change); SEER, Surveillance, Epidemiology, and End Results. Source: Negoita et al. (5). Annual report to the Nation on the status of Cancer, Part II: Recent changes in prostate cancer trends and disease characteristics.

countries, but it has revealed to be difficult due to differences in data collection and detection pathways (10).

Looking at the 1975–2017 data provided by the NCHS, we can see that there has been a slow increase in mortality since 1987, with an annual percent change (APC) of 0.9, reaching its peak between 1987 and 1991 with an APC of 3 for all races and 3.1 for white men. This peak was slightly delayed for black men, peaking in 1988 (APC 3.3). The highest mortality for all races was observed between 1975 and 2015, especially in 1993 (39.3 per 100,000 people). Again, mortality for black men reached its highest point in 1993 (81.9 per 100,000 people), two years after the peak for white men (36.5 per 100,000 people) (5). Since then, a steady decrease in mortality was observed: 1991–1994 (APC –0.5); 1994–1998 (APC –4.2); 1998–2013 (APC –3.5); and 2013–2017 (APC –0.3). A higher decline



	ALL RACES COMBINED	NON-HISPANIC WHITE	NON-HISPANIC BLACK	ASIAN/PACIFIC ISLANDER	AMERICAN INDIAN/ALASKA NATIVE	HISPANIC
Incidence	104.1	97.1	173.0	52.9	68.0	86.8
Mortality	19.1	18.0	38.7	8.6	18.7	15.7

Figure 3. Prostate cancer incidence and mortality Rates. By race and ethniciy, United States, 2012 to 2017. Source: Siegel et al. (19) Cancer Statistics 2020.

in mortality was observed in black men (APC –2.5) compared to white men (APC –0.7). During 2001–2015, the rate of mortality decreased among black men with an APC of –4.2 (5).

PROSTATE CANCER IN THE AMERICAS OTHER THAN THE USA

Canada showed an increase in crude prostate cancer incidence/diagnosis rates during 1992–2010 by 1.70 ± 0.30 cases per 100,000 males per year. However, if the data are age-adjusted, no significant increase in incidence rates were noted. Over the same period Canada witnessed 69,655 deaths, with an overall decline. Crude mortality rates reduced by –0.19 ± 0.022 deaths per 100,000 males per year between 1992 and 2010, significantly lower in the quintile with the highest percentage of African–Canadian/Black individuals (11).

Columbia, Costa Rico, and Ecuador witnessed an increase in prostate cancer incidence during 1993–2002. Incidence rates in Brazil have been relatively high but remained stable in more recent years, following an increase in 2012 (12). According to GLOBOCAN 2008, the incidence of prostate cancer in South America was 50.2 cases per 100,000. These figures rose to 59.2 in 2020 (12, 13). Mortality trends in the Americas are difficult to analyze due to the heterogeneity and lack of data available. Rates are increasing in Brazil, Colombia and Ecuador, decreasing in Argentina, Costa Rica and Chile, and stable in Mexico (12). The lowest mortality was observed in Brazil and Mexico (12–13 per 100,000 people) in 2000. In 2020, the overall mortality rate for prostate cancer in the Americas was 14.2 cases per 100,000 people a slight reduction when compared to the 16.2 case per 100,000 observed in 2008 (12, 13). Unfortunately, due to the lack of resources, no regular screening plans are active in south America with the exception of the Barretos Cancer Hospital in Brazil, which has a program that offers screening for prostate cancer (PSA and DRE) as well as screening for other common types of cancer (ie, skin, breast, and cervix) using mobile units in 231 municipalities from 6 states. From January 2004 to December 2007, 17,571 men, 45-years-old,were screened and 652 prostate cancer cases were identified, mostly with localized disease (93.4%).

PROSTATE CANCER IN CENTRAL AMERICA/CARIBBEAN

The Caribbean has one of the highest rates of prostate cancer worldwide. Incidence rates have been registered as high as 304 cases per 100,000 (14–16). Hennis *et al.* reported an overall crude incidence rate of 131.0 (95% CI: 123.4– 139.0) per 100,000 men; when data were standardized to the US, European, or World populations, this was 160.4 (95% CI: 151.0– 170.2), 163.1 (95% CI: 153.4–173.3), and 112.0 (95% CI: 105.2–119.3) per 100,000 men (17). Stratifying by age, it was noted that, prostate cancer incidence increased from 6.0 (95% confidence interval: 1.6–15.3) per 100,000 men at ages 40 to 44 years to 1,026.6 (95% CI: 898.8–1,167.6) per 100,000 in men aged 70 to 74 years, and declined thereafter (17). In the paper, it was also noted that, differently from the USA, where socioeconomic and health-care access issues need to be considered, Barbados provides free access to healthcare. However, it must be taken into consideration that the information provided by the Public Health services of the area are often imprecise and sparse, and therefore it is particularly challenging to generate and provide reliable data. For the period 2003–2007, Gibson and Gibson reported that the Jamaican age-standardised rate (ASR) for prostate cancer was 78.1 per 100,000 persons which is a substantial increase when comparted to 65.5 per 100,000 people of the period 1998–2002 (15). Prostate cancer mortality in Trinidad and Tobago has been registered among the highest in the world, with an annual increase in mortality by 4.5% over the last decade (12). For Barbadian men, mortality ranged from 63.2 to 101.6 per 100,000 persons between 1995–2008 (17). In 2020, the IARC (International Agency for Research on Cancer) provided similar figures to those of Gibson and Gibson with an ASR incidence of 75.8 cases per 100,000 people, whereas the mortality was 27.9 cases per 100,000 people (13).

Among the Central American countries, Costa Rica leads with an ASR (age standardised to World Population rate) of 53.8 cases per 100,000 people, followed by Mexico and Cuba with 28.9 and 24.3 cases per 100,000 people respectively (18). Unfortunately, epidemiological data are scarse for those regions. Trends are available only for Costa Rica which showed an annual increase in incidence of 3.8% per annum over the period 1997–2008. ASR on mortality put Belize at first place with 28.9 cases per 100,000 people followed by Cuba and Mexico with 24.1 and 17.0 cases per 100,000 people for the period 2003–2010, respectively. Costa Rica stops at 14.8 cases per 100,000 people (19). The most recent data on Central America published by GLOBOCAN 2020 shows an ASR incidence of 43.8 and mortality of 11.0 cases per 100,000 people (13). No active screening program are currently in place in Central America. Between 2004 and 2006, in Monterrey (Mexico) a screening program was run, using PSA and DRE; screening of 973 men, 40 years of age, showed that only 44% (55/125) of the men who had an abnormal result underwent prostate biopsy, and 27% (15/55) of these were diagnosed with prostate cancer, mostly with high grade lesions (based on Gleason scores 7) (18).

PROSTATE CANCER IN AFRICA

The incidence of prostate cancer in Africa is belived to be high. As it has been shown in many publications, prostate cancer is the leading cancer in terms of incidence and mortality in men of African origin. However, data is fragmented and incomplete. According to Echinemane *et al.* prostate cancer is more common than liver cancer, non-Hodgkin's lymphoma, and lung cancer in Abidjan, Ivory Coast (20). In contrast, Chu *et al.* demonstrated that prostate cancer incidence among African Americans was as much as 40 times higher than black men in Africa (21). The highest rates were reported in East Africa with figures of 10.7–38.1 per 100,000 people and the lowest rates were reported in West African countries, at 4.7–19.8 per 100,000 people. However, data collection and PSA testing are both minimal in the continent, thus severely affecting the reliability and statistical analysis of the data.

Prostate cancer is becoming more and more an issue of public concern in Africa due to the fact that the majority of new diagnoses are advanced/metastatic cancers, with poor prognosis and low chances of long-term survival. About 64% of new prostate cancer cases in a Nigerian hospital had advanced disease and died within two years of diagnosis. Those figures are dismal when compared to the American data. For comparison, in the United States, 66% of the patients who had a diagnosis of prostate cancer in 1975 survived more than 5 years while between 2008 and 2014 that number rose to 98.2% (22). In 2010, Ferlay *et al*. estimated that 57,048 deaths will be caused by prostate cancer in Africa by 2030. This represents a 104% increase over the next 10 years (23). According to the data provided by the IARC 2020, ASR of incidence and mortality for prostate cancer in the African continent were respectively 29.7 and 16.3 cases per 100,000 people.

PROSTATE CANCER IN EUROPE

Due to the availability of more comprehensive information, data from the United Kingdom (UK) are discussed separately from the other European countries.

United Kingdom

Currently, PSA screening is not offered in UK as part of early prostate cancer detection. Cancer Research UK advises that men 50-years or older can request screening through their general practitioner , where a discussion about risks and benefits will be made. According to the most recent data, prostate cancer is the most common cancer diagnosed in men in the UK, representing 26% of all new diagnoses in 2017 (24). For males aged 45 years and over, prostate cancer was the most common cancer, peaking at 32.8% of all cancers in the 65–74 age group. Considering age-specific incidence, prostate cancer rates rise steeply from around age 50–54, peak in the 75–79 age group before dropping slightly and remaining stable in the oldest age groups. The highest figures are in the 75 to 79 age group as shown in Figure 3. Between 1993–1995 and 2015–2017, the European age standardised incidence rates of prostate cancer increased by 41%, with a 4% increase between 2005–2007 and 2015–2017. The percentage increase for each age group is: 50–59 (291%), 60–69 (137%), and 70–79 (41%). In contrast, in the oldest group (over 80), the incidence decreased by 28%. These numbers are likely secondary to random findings due to PSA testing, incidental detection of asymptomatic disease and the increase of benign prostatic hyperplasia procedures such as transurethral resection of the prostate (TURP) and its histological findings (24). An important factor that must be taken into account is the ethnic heterogeneity of prostate cancer across the UK population. According to "The PROCESS Cohort Study" published in 2008, Afro-Caribbean men had an age-adjusted prostate cancer incidence rate of 173 per 100,000 people compared to a rate of 56.4 per 100,000 for UK white men and 139 for black African men. Moreover, it was found that Afro-Caribbean men residing in the UK were three times more likely to be diagnosed with the disease and were diagnosed 5 year earlier than Caucasian men residing in the UK, despite both groups having equal access to diagnostic services (25). In conclusion, the lifetime risk of being diagnosed with prostate cancer is 13.2–15.0% for white males, while in black males it is significantly higher (23.5–37.2%), and in Asian males it is significantly lower (6.3–10.5%) (26).

During the 1990s, PSA testing was introduced; it showed a 10-fold difference in uptake in the UK compared to the USA. For Comparison, in 2001, in the USA, 57% of men aged 50 years or older reported having a PSA test within the previous 12 months. By contrast, for each year between 1999 and 2002, an estimated 6% of men aged 45–84 years were tested in the UK (27).

Despite those differences, Colling et al. showed that although age-adjusted prostate cancer mortality reached its maximum in early 1990s at almost identical rates, there was not a similar decrease in mortality in the UK that was seen in the USA. From the mid-1990s, Age-adjusted prostate-cancer mortality declined in the USA by 4.17% each year between 1994 and 2004, almost four-times the rate of decline in the UK (1.14% each year), especially in patients aged 75 years or

older (27). This data may be explained by a general tendency in the USA to treat prostate cancer more aggressively than in the UK. The most recent figures (2016–2018) showed that every year in UK, around 11,900 men die of prostate cancer. Those figures represented 13% of all cancer deaths in males in the UK in 2018 (24). However, as can be seen from Figure 4 over the last decade (between 2006–2008 and 2016–2018), prostate cancer age-standardized mortality rates for males decreased by 10%. According to the data provided by Cancer Research UK, mortality rates for prostate cancer are projected to fall by 16% in the UK between 2014 and 2035, to 48 deaths per 100,000 males by 2035.

Prostate cancer in other European countries

A general increase in prostate cancer has been witnessed in Western Europe (28). It is unclear if these figures are secondary to PSA screening or other factors such as diet and low exposure to sunlight (Vitamin D) (29). Since mid 1990, an increase of APC between 4 and 5% has been witnessed in Austria, France, and Switzerland. Figures remained stable in other countries such as Netherlands from 1999 to 2008. According to Center *et al*. (12), mortality rates decreased in Austria, France, Switzerland, Germany and Netherlands. The main decline was seen in Austria (APC 4.0) whereas the lowest in Germany and Netherlands (APC 2.3). In Southern Europe, an overall increase in prostate cancer was recorded between

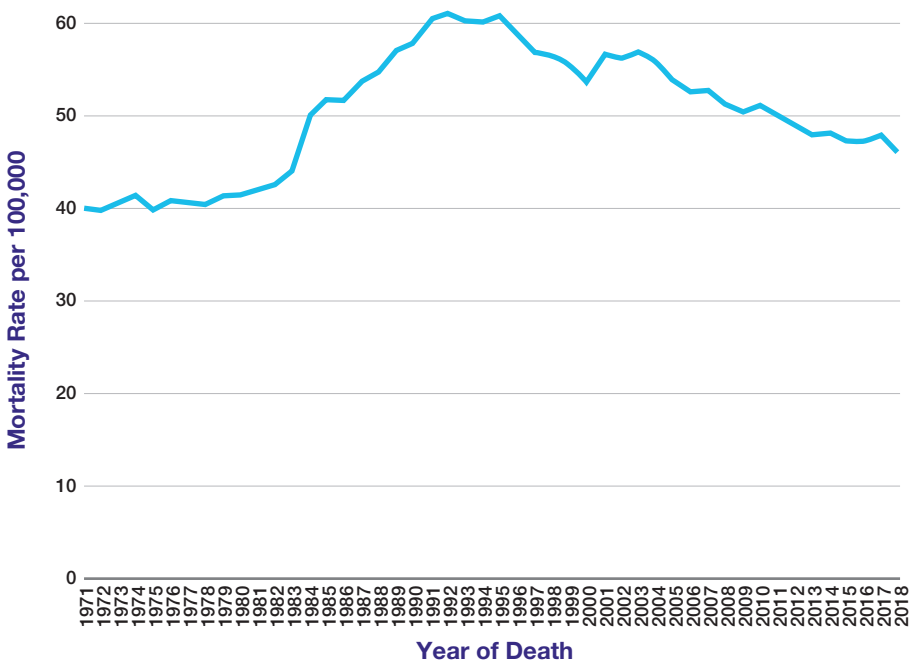


Figure 4. Prostate cancer moratlity. European age-standardised mortality rates per 100,000 population, Males, UK, 1971–2018. Source: https://www.cancerresearchuk.org [accessed on 06 April 2021].

1998 and 2007. The largest increase was seen in Croatia (APC 8.5%) followed by Italy, Slovenia, Malta and Spain. Mortality rates were more heterogenous with decline seen in Italy, Malta, Spain and increase in Croatia and Slovenia (28). An increase in prostate cancer incidence was recorded over the past decade in four out of the five Nordic countries (Denmark, Iceland, Norway, and Sweden). The most relevant increase was in Denmark (APC 8.2% between 1999 and 2008). Finland showed stable figures. In terms of mortality, a stable trend was seen in Denmark and Iceland whereas Norway and Sweden experienced a substantial decrease. In Finland, a decrease of 3.1% per annum has been seen since 2000 (12, 28).

PROSTATE CANCER IN RUSSIA AND FORMER SOVIET UNION COUNTRIES

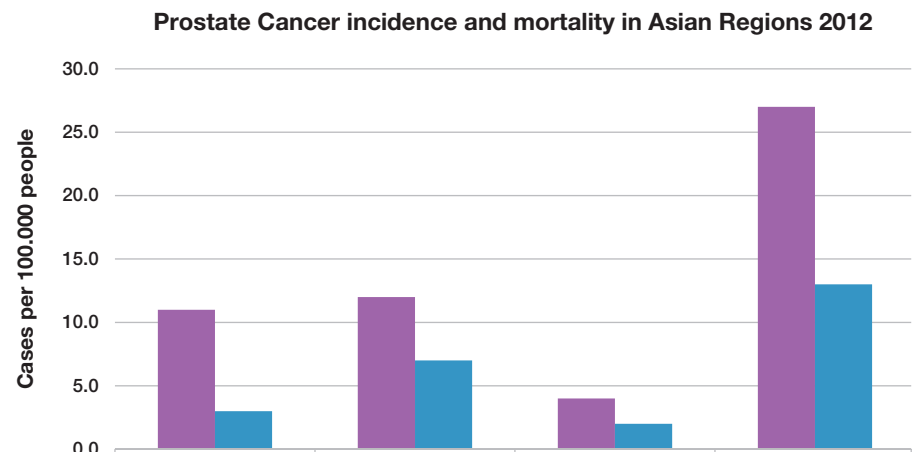
This section analyses the incidence and mortality of prostate cancer in the Baltic States, Belarus, Russian Federation and Ukraine. It should be pointed out that these countries have very different profiles of PSA testing uptake. The Russian Federation was the first to introduce PSA testing in the 1990. Since 2013, PSA is part of a national health check-up program. Lithuania introduced the test in 2000. A nationwide PSA screening program has been introduced since 2006. By 2010 around 72–78% of the total eligible male population received a PSA test. In Latvia, PSA testing has not been funded by the Government. A recent review by Patasius et al. showed that the countries can be divided in two groups (30): high (>100 cases per 100,000 people), and low (<100 cases per 100,000 people) incidence countries. The Baltic States (Estonia, Latvia, and Lithuania) belong to the former whereas Belarus, the Russian Federation and Ukraine to the latter. Lithuania is the country with the highest incidence overall (203.4 per 100,000 people) followed by Estonia and Latvia (158.3 and 102.2 per 100,000 people, respectively). In the Russian Federation, figures are low (55.2 per 100,000 people) as it is for Ukraine (36.2 per 100,000 people). A recent study showed that the APC of prostate cancer has been constantly positive for all years; however, the inconsistency of cancer databases (1978 to 2016 in Lithuania and 2000–2012 in Ukraine) should be taken into account. The APC ranges from 3.4 to 7.4 for Ukraine and Lithuania, respectively. In Estonia and Lithuania, incidence peak for men aged 50–74 was in 2007, followed by an incidence decrease in Lithuania since 2007 and Estonia since 2011.

Despite remarkable differences in incidence, mortality rates among all countries are relatively similar. The highest figures were recorded in Lithuania (ASR of 26.68, 1995–1999) and the lowest in the Russian Federation (ASR of 12.24 in the same period). Age-specific mortality was the highest in the over 85 age group. During 2011–2015, the mortality rates in this group doubled. However, the relative difference between the Baltic states and other countries remained unchanged (30). The reason behind the high mortality rates after the implementation of the PSA-based screenings is unclear. It is possible that these figures are secondary to over-reporting of prostate cancer as the underlying cause of death in death certificates. A similar issue was noted in the USA in 1991, a few years after the implementation of PSA screening (31).

PROSTATE CANCER IN ASIA

The incidence of prostate cancer in Asian countries has been historically much lower than their Western counterpart, ranging between 4.5 cases per 100,000 persons for South-Central Asia, 10.5 for Eastern Asia and 11.2 for Southeast Asia (12). Those values could be explained both by a low susceptibility of Asian men to prostate cancer and the lack of a systematic screening program. However, there is evidence that these figures are increasing in several countries (23). A review by Ha Chung et al., showed a general increase in prostate cancer incidence across China, India, South Korea, Vietnam, Japan, and Singapore (32). These figures were supported by data from GLOBOCAN 2008 and 2012 (23, 32). Sim and Cheng noted that in some centres in Japan, the incidence rate rose from 6.3 to 12.7 (102% increase) between 1978 and 1997, while the incidence rates in Singaporean Chinese men increased to 118% (from 6.6 to 14.4 case per 100,000 people) within the same period (33). The lowest incidence reported in Asia was in Shanghai whereas the highest was in the Rizal Province in the Philippines. Figure 5 shows the differences in incidence and mortality across Asia. Studies have also shown that Asian Men living in the United States develop higher risk of prostate cancer than their counterparts living in Asia suggesting that change in lifestyle, and probabaly increased screening, could be the major contributors (34).

In 2008, prostate cancer accounted for about 2% of all cancer-associated deaths in the Asia-Pacific. In the same period, over 250,000 men died of prostate cancer around the globe, of which, 42,000 (16%) deaths occurred in the



	Eastern Asia	South-East Asia	South-Central Asia	Western Asia
Incidence	11.0	12.0	4.0	27.0
Mortality	3.0	7.0	2.0	13.0

Figure 5. Incidence and mortality rates (per 100,000) in different Asian regions in 2012. Data source: GLOBOCAN 2012 v1.0, Cancer Incidence and Mortality Worldwide: IARC Cancer Base No. 11. Source: Adapted from Chen et al., 2014, "Prostate Cancer in Asia: A Collaborative Report."

Asia-Pacific area. China accounted for 34% of prostate cancer deaths, Japan 24%, and Indonesia 16% (34). In addition, it was noted that in Asian countries, despite having a 20 times lower incidence rate than USA, prostate cancer mortality was only about 2.5% lower than the USA. A useful tool that can be used is the mortality rate to incidence rate ratio, MR/IR. In 2012, the MR/IR in Asia was between 0.3 and 0.6 whereas it was 0.1 in the USA and 0.18 in Europe (35). This disparity between IR and MR could be explained by the lack of screening tools and the consequent delayed diagnosis in Asia compared to Western countries. In view of these results, Zhang *et al*. suggested that it would be wise and useful to integrate PSA screening, as well as develop a nationwide cancer registration system (35).

PROSTATE CANCER IN OCEANIA AND NEW ZEALAND

Australia approved the use of PSA in 1989, similarly to the USA. The introduction of this test caused a steep increase in the lifelong risk of detecting prostate cancer from the 6.1% of 1982 (1 in 17) to 18.4% of 1994 (36). In 1996, the Australian Health Advisor Committee recommended against screening asymptomatic men for prostate cancer. Between 2008 and 2009, 21–25% of the Australian men aged 50–75 had a PSA test (37). In 2012, the introduction of the American USPSTF recommendation against PSA testing caused a further reduction in screening. The impact of the 2018 USPSTF has not been studied yet in the country. Overall, the lifetime risk of prostate cancer has shown some fluctuations after the years, but has never dipped below 15%, and in 2012 it was 19.7% (95% CI 19.4% to 20%: 1 in 5) (36). According to IARC 2008, the prostate cancer ASR incidence was 94.5 per 100,000 persons, which dropped to 70 per 100,000 in 2020 (1). Mortality rates decreased in Australia by 2.3% between 1995 and 2004 and by 2.8% per year on average in New Zealand (12). According to GLOBOCAN, the ASR mortality secondary to prostate cancer was 15.3 cases per 100,000 people in 2008 (12) and 15.3 per 100,000 people in 2020 (1).

CONCLUSION

Prostate cancer is one of most common cancers diagnosed worldwide. With more than 1,400,000 (>7%) new cases and more than 375,000 (3.8%) deaths annually, it is the third most common malignancy diagnosed, and is the eighth cause of cancer death. Its incidence varies greatly between regions of the World, and is hugely affected by PSA testing and related screening programs.. It has been demonstrated that wherever screening is available, the incidence increases without necessarily seeing associated steady decrease in cancer specific mortality. Currently, there is no international consensus on the best approach on PSA testing. It is well known and documented that men of African origin are more prone to develop prostate cancer over the course of their life, whereas Asian ethnicities seems to be less affected. The reason behind those differences is still unclear, but is likely to be multifactorial.

Conflict of interest: The author declares no potential conflicts of interest with respect to research, authorship and/or publication of this article.

REFERENCES

1. Sung H, Ferlay J, Siegel RL, Laversanne M, Soerjomataram I, Jemal A, et al. Global cancer statistics 2020: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA: a cancer journal for clinicians. 2021. https://doi.org/10.3322/caac.21660
2. Baade PD, Youlden DR, Krnjacki LJ. International epidemiology of prostate cancer: geographical distribution and secular trends. Mol Nutr Food Res. 2009;53(2):171–84. https://doi.org/10.1002/mnfr.200700511
3. Surveillance E, and End Results (SEER) Mortality - All COD, Aggregated With State, Total U.S. (1969–2017) <Katrina/Rita Population Adjustment>, National Cancer Institute, DCCPS, Surveillance Research Program, released December 2019. Underlying mortality data provided by NCHS (www.cdc.gov/nchs) [Accessed on 06 April 2021].
4. Potosky AL, Miller BA, Albertsen PC, Kramer BS. The role of increasing detection in the rising incidence of prostate cancer. JAMA. 1995;273(7):548–52. https://doi.org/10.1001/jama.1995.03520310046028
5. Negoita S, Feuer EJ, Mariotto A, Cronin KA, Petkov VI, Hussey SK, et al. Annual Report to the Nation on the Status of Cancer, part II: Recent changes in prostate cancer trends and disease characteristics. Cancer. 2018;124(13):2801–14. https://doi.org/10.1002/cncr.31549
6. Siegel RL, Miller KD, Jemal A. Cancer statistics, 2016. CA: a cancer journal for clinicians. 2016;66(1):7–30. https://doi.org/10.3322/caac.21332
7. Moyer VA, Force USPST. Screening for prostate cancer: U.S. Preventive Services Task Force recommendation statement. Ann Intern Med. 2012;157(2):120–34. https://doi.org/10.7326/0003-4819-157-2-201207170-00459
8. Bibbins-Domingo K, Grossman DC, Curry SJ. The US Preventive Services Task Force 2017 Draft Recommendation Statement on Screening for Prostate Cancer: An Invitation to Review and Comment. JAMA. 2017;317(19):1949–50. https://doi.org/10.1001/jama.2017.4413
9. Fenton JJ, Weyrich MS, Durbin S, Liu Y, Bang H, Melnikow J. Prostate-Specific Antigen-Based Screening for Prostate Cancer: Evidence Report and Systematic Review for the US Preventive Services Task Force. JAMA. 2018;319(18):1914–31. https://doi.org/10.1001/jama.2018.3712
10. Kheirandish P, Chinegwundoh F. Ethnic differences in prostate cancer. Br J Cancer. 2011;105(4):481–5. https://doi.org/10.1038/bjc.2011.273
11. Lagace F, Ghazawi FM, Le M, Savin E, Zubarev A, Powell M, et al. Incidence and Mortality of Prostate Cancer in Canada during 1992–2010. Curr Oncol. 2021;28(1):978–90. https://doi.org/10.3390/curroncol28010096
12. Center MM, Jemal A, Lortet-Tieulent J, Ward E, Ferlay J, Brawley O, et al. International variation in prostate cancer incidence and mortality rates. Eur Urol. 2012;61(6):1079–92. https://doi.org/10.1016/j.eururo.2012.02.054
13. IARC. Estimated age-standardized incidence rates (World) in 2020, worldwide, all ages. 2020.
14. Bunker CH, Patrick AL, Konety BR, Dhir R, Brufsky AM, Vivas CA, et al. High prevalence of screening-detected prostate cancer among Afro-Caribbeans: the Tobago Prostate Cancer Survey. Cancer Epidemiol Biomarkers Prev. 2002;11(8):726–9.

15. Gibson TN, Hanchard B, Waugh N, McNaughton D. Age-specific incidence of cancer in Kingston and St. Andrew, Jamaica, 2003–2007. West Indian Med J. 2010;59(5):456–64.
16. Glover FE, Jr., Coffey DS, Douglas LL, Cadogan M, Russell H, Tulloch T, et al. The epidemiology of prostate cancer in Jamaica. J Urol. 1998;159(6):1984–7. https://doi.org/10.1016/S0022-5347(01)63220-8
17. Hennis AJ, Hambleton IR, Wu SY, Skeete DH, Nemesure B, Leske MC. Prostate cancer incidence and mortality in barbados, west indies. Prostate Cancer. 2011;2011:565230. https://doi.org/10.1155/2011/565230
18. Sierra MS, Soerjomataram I, Forman D. Prostate cancer burden in Central and South America. Cancer Epidemiol. 2016;44 Suppl 1:S131-S40. https://doi.org/10.1016/j.canep.2016.06.010
19. Siegel RL, Miller KD, Jemal A. Cancer statistics, 2020. CA: a cancer journal for clinicians. 2020;70(1):7–30. https://doi.org/10.3322/caac.21590
20. Echimane AK, Ahnoux AA, Adoubi I, Hien S, M'Bra K, D'Horpock A, et al. Cancer incidence in Abidjan, Ivory Coast: first results from the cancer registry, 1995–1997. Cancer. 2000;89(3):653–63. https://doi.org/10.1002/1097-0142(20000801)89:3<653::AID-CNCR22>3.0.CO;2-Z
21. Chu LW, Ritchey J, Devesa SS, Quraishi SM, Zhang H, Hsing AW. Prostate cancer incidence rates in Africa. Prostate Cancer. 2011;2011:947870. https://doi.org/10.1155/2011/947870
22. Howlader N, Noone A, Krapcho M, Miller D, Bishop K. SEER Cancer Statistics Review 1975–2013. National Cancer Institute, Bethesda MD; 2016.
23. Ferlay J, Shin HR, Bray F, Forman D, Mathers C, Parkin DM. Estimates of worldwide burden of cancer in 2008: GLOBOCAN 2008. Int J Cancer. 2010;127(12):2893–917. https://doi.org/10.1002/ijc.25516
24. England PH. Cancer registration statistics, England: 2017. 2019.
25. Ben-Shlomo Y, Evans S, Ibrahim F, Patel B, Anson K, Chinegwundoh F, et al. The risk of prostate cancer amongst black men in the United Kingdom: the PROCESS cohort study. Eur Urol. 2008;53(1):99–105. https://doi.org/10.1016/j.eururo.2007.02.047
26. Lloyd T, Hounsome L, Mehay A, Mee S, Verne J, Cooper A. Lifetime risk of being diagnosed with, or dying from, prostate cancer by major ethnic group in England 2008–2010. BMC Med.. 2015;13:171. https://doi.org/10.1186/s12916-015-0405-5
27. Collin SM, Martin RM, Metcalfe C, Gunnell D, Albertsen PC, Neal D, et al. Prostate-cancer mortality in the USA and UK in 1975–2004: an ecological study. Lancet Oncol. 2008;9(5):445–52. https://doi.org/10.1016/S1470-2045(08)70104-9
28. Taitt HE. Global Trends and Prostate Cancer: A Review of Incidence, Detection, and Mortality as Influenced by Race, Ethnicity, and Geographic Location. Am J Mens Health. 2018;12(6):1807–23. https://doi.org/10.1177/1557988318798279
29. Mottet N, Bellmunt J, Bolla M, Briers E, Cumberbatch MG, De Santis M, et al. EAU-ESTRO-SIOG Guidelines on Prostate Cancer. Part 1: Screening, Diagnosis, and Local Treatment with Curative Intent. Eur Urol. 2017;71(4):618–29. https://doi.org/10.1016/j.eururo.2016.08.003
30. Patasius A, Innos K, Barchuk A, Ryzhov A, Leja M, Misins J, et al. Prostate cancer incidence and mortality in the Baltic states, Belarus, the Russian Federation and Ukraine. BMJ Open. 2019;9(10):e031856. https://doi.org/10.1136/bmjopen-2019-031856
31. Feuer EJ, Merrill RM, Hankey BF. Cancer surveillance series: interpreting trends in prostate cancer--part II: Cause of death misclassification and the recent rise and fall in prostate cancer mortality. J Natl Cancer Inst. 1999;91(12):1025–32. https://doi.org/10.1093/jnci/91.12.1025
32. Ha Chung B, Horie S, Chiong E. The incidence, mortality, and risk factors of prostate cancer in Asian men. Prostate Int.. 2019;7(1):1–8. https://doi.org/10.1016/j.prnil.2018.11.001
33. Sim HG, Cheng CW. Changing demography of prostate cancer in Asia. Eur J Cancer. 2005;41(6):834–45. https://doi.org/10.1016/j.ejca.2004.12.033
34. Baade PD, Youlden DR, Cramb SM, Dunn J, Gardiner RA. Epidemiology of prostate cancer in the Asia-Pacific region. Prostate Int. 2013;1(2):47–58. https://doi.org/10.12954/PI.12014
35. Zhang L, Yang BX, Zhang HT, Wang JG, Wang HL, Zhao XJ. Prostate cancer: an emerging threat to the health of aging men in Asia. Asian J Androl. 2011;13(4):574–8. https://doi.org/10.1038/aja.2010.126

36. Pathirana T, Hayen A, Doust J, Glasziou P, Bell K. Lifetime risk of prostate cancer overdiagnosis in Australia: quantifying the risk of overdiagnosis associated with prostate cancer screening in Australia using a novel lifetime risk approach. BMJ Open. 2019;9(3):e022457. https://doi.org/10.1136/bmjopen-2018-022457
37. Zargar H, van den Bergh R, Moon D, Lawrentschuk N, Costello A, Murphy D. The impact of the United States Preventive Services Task Force (USPTSTF) recommendations against prostate-specific antigen (PSA) testing on PSA testing in Australia. BJU Int. 2017;119(1):110–5. https://doi.org/10.1111/bju.13602

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The Etiology of Prostate Cancer

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Doi: https://doi.org/10.36255/exonpublications.prostatecancer.etiology.2021

Abstract: While the exact etiology of prostate cancer remains elusive, various modifiable and unmodifiable risk factors have been suggested as contributing factors. These include age, ethnicity, family history, genetics, obesity, diet, hormones, smoking, alcohol, and certain medications; however, none of these, perhaps with the exception of ethnicity and age, has been conclusively proven to be a definite etiological factor for prostate cancer. Men of black African ancestry are more prone to the disease. The probability of developing prostate cancer increases with age, from 0.005% in men younger than 39 years of age to 2.2% in men between 40 and 59 years, and 13.7% in men between 60 and 79 years. A better understanding of the environmental, genetic, nutritional, hormonal, and molecular landscape that shape the etiology and pathophysiology of prostate cancer will lead to better preventative strategies, enhanced diagnostic pathways, and improved management of the disease. This chapter provides an overview of the current understanding of the etiology of prostate cancer.

Keywords: androgens; ethnicity; family history; prostate cancer; risk factors

In: *Prostate Cancer*. Bott SRJ, Ng KL (Editors). Exon Publications, Brisbane, Australia. ISBN: 978-0-6450017-5-4; Doi: https://doi.org/10.36255/exonpublications.prostatecancer.2021

INTRODUCTION

Prostate cancer is the most common cancer in men and is the third leading cause of cancer-related mortality after lung and colorectal cancers in Europe (1). The etiology of prostate cancer is multifactorial and remain quite baffling, with numerous modifiable and unmodifiable risk factors associated with its development. Some well-established risk factors include advanced age, positive family history, and African ancestry. However, epidemiological studies based on geographical distribution and ethnic variability of prostate cancer have suggested that environmental factors, lifestyle, and diets can influence the risk of prostate cancer and its progression. Global variations in prostate cancer incidence are well known and is comprehensively discussed in chapter 1 of this book. The high incidence of prostate cancer can also be attributed to the increasing use of prostate specific antigen (PSA) screening, aging population, and better diagnostic modalities. Understanding the etiology, pathophysiology, and natural history of prostate cancer is useful to aid in the diagnosis and better management of this cancer.

AGE

Age is a well-established risk factor for prostate cancer. Incidence of prostate cancer increases with age. Prostate cancer is rare below the age of 40. This age-related trend is seen globally, both in developed and developing countries (2). PSA screening has led to an earlier age of prostate cancer detection as it has a lead time of approximately 10 years before any symptoms occur. The probability of developing prostate cancer increases from 0.005% in men younger than 39 years of age to 2.2% in men between 40 and 59 years, and 13.7% in men between 60 and 79 years (3). The probability of histological diagnosis of prostate cancer is higher with 50% of men between 70 and 80 years of age showing histological evidence of malignancy (4). Fortunately, majority of these low grade, low volume histological diagnosis of prostate cancer follows an indolent course without any significant risk of dying from the disease.

FAMILY HISTORY AND GENETIC PREDISPOSITION

Prostate cancer has an increased heritability. Men with a brother or father diagnosed with prostate cancer have a two to four-fold risk of developing prostate cancer—the risk is higher if a brother is diagnosed (5). The risk attributed to genetic factors increases further with more relatives being affected and earlier age of diagnoses (6). In addition, the Nordic Twin Study of Cancer estimated that the variation of prostate cancer risk among twins attributed to genetic factors was 57%, thus making prostate cancer one of the most heritable cancers (7). Recent studies have also suggested increased risk of prostate cancer in families with familial breast cancer and familial prostate cancer traits. A large prospective study of 37002 men for 16 years in the USA identified that those with familial breast cancer had a 21% greater risk of prostate cancer overall; family history of prostate

cancer alone was associated with a 68% increased risk of total disease and those who were with a family history of both cancers were also at elevated risk (8). One possible explanation for the link between familial breast cancer and increased prostate cancer risk lies in the BRCA gene mutation. Given the link between BRCA and breast cancer, and evidence of increased prostate cancer risk among male BRCA carriers, inherited BRCA mutations may provide one biological mechanism for familial clustering of prostate and breast cancer (6). These results from familial history links further enhance and confirm the role of genetic predisposition from susceptible genes associated with prostate cancer risk which has led to the genome wide association studies (GWAS) in prostate cancer. GWAS have provided greater insight to the genetic predisposition for prostate cancer risk. There are more than 180 independent single nucleotide polymorphisms (SNPs) associated with prostate cancer risk, which account for a third of familial prostate cancer heritability risk (9, 10). A review by Benafif *et al.* (11) showed that prostate cancer genetic susceptibility variants can explain 37.5% of the familial relative risk of prostate cancer, with approximately 6% accounting for rare variants (including 2 rare SNPs on 8q24 and HOXB13) and 31.5% for commonly occurring SNPs. In the largest prostate cancer GWAS and meta-analysis reported by Schumacher et al., 63 novel prostate cancer susceptibility loci were identified bringing the total number of known loci to 167 (12). Armed with this genetic information, better targeted prostate cancer screening programs aimed at those at higher risk groups can be performed utilizing genetic identity information kits. Such screening program can target those who will be at risk of clinically significant prostate cancer and will be able to replace the non-specific PSA screening and avoid unnecessary prostate biopsies. Once prostate cancer is diagnosed, the affected individual genetic profiles can be utilized to predict aggressiveness and progression of disease. Furthermore, individualized targeted therapy can be prepared based on these genetic tumor profiles to enhance the precision of the treatment regimen.

ETHNICITY

There are ethnic and geographic variations in the incidence of prostate cancer. There is a higher incidence, severity, and mortality rates amongst men of black African descent. In the USA, there is a threefold variability amongst different ethnic/racial groups, with the highest incidence amongst black men of African descent (13). Mortality rates are 2.4 times higher in black men in the USA when compared to white men (14). One observation that could account for these differences in incidence and mortality is the prevalence of multiple prostate cancer genetic risk loci across racial/ethnic groups (15). A review by Rani *et al.* explained the observations of lower TMPRSS-ERG fusion, PTEN deletion, differential methylation of genes (SNRPN, SHANK2, MST1R, and ABCG5), and up-regulation of MNX1 in men of African descent promoted oncogenesis due to the deletion of such protective tumor suppressor roles. Another chemokine receptor, DARC, found in red blood cells where they remove chemokines from prostate tumor microenvironment, has been shown to be depleted in large proportion of African men, contributing to increased incidence and mortality rates in this ethnic group (16).

SMOKING AND ALCOHOL

Among the modifiable risk factors for prostate cancer, smoking has been shown to have an association with prostate cancer incidence and mortality. A meta-analysis of 24 cohort studies by Huncharek *et al.* revealed that there was no increased risk or incidence of prostate cancer among current smokers, but the risk increased with increasing amount smoked. Furthermore, ex-smokers had increased risk of prostate cancer and heavy smokers had a 24–30% increase risk of prostate cancer related deaths (17). Previous studies have not been conclusive regarding alcohol consumption and prostate cancer risk. However, a systematic review and meta-analysis of 340 studies noted that there was a significant dose-response relationship between alcohol consumption and prostate cancer risk. The risk increases with increasing volume of alcohol intake when compared to non-drinkers (18). This relationship has implications affecting public health strategies which may reduce the risk of prostate cancer in developed countries.

OBESITY AND METABOLIC SYNDROME

Obesity and increased body mass index have been associated with numerous cancers including prostate cancer, with increased adiposity leading to increased mortality risk of prostate cancer (19). According to the meta-analysis by Cao and Ma, an increase in 5kg/m^2 in body mass index led to a 20% higher risk of prostate cancer mortality (20). Despite that, the underlying mechanisms as to why this is the case remains elusive. Three possible reasons which relate the risk of prostate cancer and obesity are insulin like growth factor 1 (IGF-1), sex hormones, and adipokines (21). Recently, more studies on adipokines, which are chemokines secreted by adipocytes into plasma, have shed some light in this aspect. One of the adipokines is adiponectin which has been studied extensively and implicated in the development and progression of prostate cancer. With increasing obesity, plasma adiponectin concentrations fall, especially in men (22). A meta-analysis by Liao *et al.*, showed that lower concentrations of adiponectin was significantly associated with a greater risk of prostate cancer, with various possible explanatory mechanisms which included anti-inflammatory, anti-proliferative, AMPK, and Wnt signaling pathways (23). Therefore, in future, adiponectin may be a potential biomarker in the diagnosis of prostate cancer.

Metabolic syndrome is a cluster of conditions that include hypertension, hyperglycemia, hypercholesterolemia/ high triglycerides, and excess body fat with increased waist circumference. Metabolic syndrome has been associated with increased risk of common cancers like colorectal and breast cancers. In prostate cancer, metabolic syndrome has been shown to have a slight association with incidence of prostate cancer (OR 1.17) and has a greater association with more aggressive disease and biochemical recurrence (24). This was supported by a study of 8122 men in the REDUCE trial which revealed that having three or more components of metabolic syndrome were significantly associated with a greater risk of higher-grade prostate cancer (25). This finding may suggest that controlling the effects of metabolic syndrome may prevent from aggressive prostate cancer progression.

PHYSICAL ACTIVITY, DIET, AND NUTRITION

Numerous studies have shown that there is an inverse relationship between physical activity and risk of progression and mortality from prostate cancer. One large study of 2705 men with prostate cancer revealed a 61% reduction in risk of prostate cancer-specific mortality in men who had at least three hours of vigorous exercise per week than those who did less than 1 hour per week (26). However, there is no concrete evidence to suggest if increased physical activity or regular physical exercises could reduce the risk of developing prostate cancer.

Diet and nutrition have been implicated in many cancers including prostate cancer. Numerous studies have investigated the association of prostate cancer and what we consume—fat intake, calcium, dairy, lycopenes, soy consumption, selenium, vitamin D, processed food, and Western diets. Consumption of highly processed foods can increase the risk of prostate cancer and conversely, intake of unprocessed/limited processed foods was associated with lower risk of prostate cancer as shown by PROtEuS study (27). This explains why Westernized diet of fast foods, that are highly processed, have a higher association with the incidence of prostate cancer than less processed foods. Regarding vegetarian diet, a recent meta-analysis which included almost 200 men with prostate cancer diagnosis, did not show any significant association between vegetarian diet and prostate cancer risk compared to a non-vegetarian diet (28). Lycopene is a red pigmented carotenoid found in tomatoes and watermelons. A systematic review by Rowles *et al.* showed that increased dietary and circulating lycopene lowers prostate cancer risk. Higher dietary intake and circulating lycopene levels corresponded to greater reduction in prostate cancer risk (29). Prostate cancer incidence is much lower in Asian countries where soy consumption is high. This has led to numerous studies on soy foods with the isoflavone levels (genistein and daidzein) and its association with prostate cancer. Conclusion from a systematic review by Applegate *et al.* showed that increased soy food consumption significantly lowers the risk of prostate cancer (30). The SELECT trial (Selenium and Vitamin E Cancer Prevention Trial) did not show any benefit of selenium and vitamin E supplementation in reducing the risk of prostate cancer but in fact increased the risk of high-grade cancer and type 2 diabetes mellitus (31). A Mendelian randomization analysis revealed that selenium supplementation did not enhance prostate cancer prevention, and may lead to increased risk of advanced prostate cancer and type 2 diabetes (32).

Dairy products which contain plenty of calcium have been studied extensively in prostate cancer. Most studies show that there is a positive association between dietary intake of dairy products and intake of high calcium with prostate cancer (33, 34). One probable mechanism for this lies in the fact that increased calcium levels will suppress the levels of 1,25 dihydroxyvitamin D (calcitriol), which is the active form of vitamin D. Calcitriol has been shown to affect cell cycle, induce apoptosis and inhibit the growth of normal prostatic epithelial cells, together with prostate cancer cell lines and primary cultures of prostate cancer cells (35). Dairy products are associated with increased levels of insulin like growth factors which has been linked to high-grade prostate cancer (36). However, the expert report from the World Cancer Research Fund on Diet and Cancer stated that calcium intake is a "probable" risk factor for prostate cancer, but the evidence for dairy products was weak and inconclusive (37).

Countries where there is higher fish consumption have recorded lower incidence of prostate cancer than countries of Western diet low in fish intake. Various studies looking at fish intake and prostate cancer progression have mixed results, some showing a reduction in risk while others showing no association (38, 39). Interestingly, Richman *et al.* showed that consumption of eggs and poultry with skin following diagnosis of prostate cancer, may increase the risk of prostate cancer progression (39).

Due to the abundance of literature on diet, nutrition, weight, exercise, and its association with prostate cancer, one can only be practical in the approach to reduce the risk of prostate cancer. Therefore, for those who are concerned with prostate cancer risk, Wilson *et al.* have recommended to stop smoking, increase physical activities, and observe a healthy weight. The authors have also recommended increasing fish and tomatoes intake while reducing dairy intake (40). The 2018 World Cancer Research Fund/American Institute for Cancer Research Cancer Prevention Recommendations and Prostate Cancer revealed that three components—limiting the consumption of sugary drinks, heavily processed foods, and alcohol—have been independently associated with lowering prostate cancer risk (41).

MEDICATIONS

Metformin has been the cornerstone of treatment of type 2 diabetes mellitus for a long time, but recent interest has shed light on its anti-neoplastic properties, particularly in prostate cancer patients. In a comprehensive review by Ahn *et al.*, there were numerous studies that showed metformin usage associated with reduction in risk of prostate cancer and progression, while other studies noted no association regarding incidence or survival. Various antineoplastic mechanisms of metformin involving numerous pathways like adenosine monophosphate-activated protein kinase (AMPK) activation, inhibition of the mammalian target of rapamycin (mTOR) activity and induction of apoptosis have been shown (42). In a recent meta-analysis of cohort studies investigating metformin use and prostate cancer risk, there was no association noted (43). Nonetheless, there are currently ongoing clinical trials investigating the use of metformin monotherapy, or in conjunction with androgen deprivation therapy, in metastatic prostate cancer patients, hoping to shed some light for the use of metformin in improving prostate cancer survival. Statins have also been implicated in delaying the progression of prostate cancer. In a large study of 11,000 prostate cancer patients in the United Kingdom, post-diagnostic patients who had statins showed a 34% lower risk of prostate cancer death (44). Furthermore, this effect was even stronger for men who were already on statins prior to diagnosis. Another retrospective study revealed that the time to progression was longer in patients who were on anti-androgen therapy and statins compared to those who were not on statins (45).

SEX AND VASECTOMY

Interestingly, the meta-analysis by Jian *et al.* showed that the risk of prostate cancer decreases by 4% for every 5 years delay in the age of first sexual intercourse.

The authors also concluded that an increment of 10 female sexual partner is associated with a 1.1-fold increase in prostate cancer risk. Moderate ejaculation frequency (2–4 times/week) was also significantly associated with lower risk of prostate cancer (46). Another aspect of ejaculation regarding vasectomy has also been implicated in prostate cancer risk. A systematic review by Bhindi *et al.* showed that there was no association between vasectomy and prostate cancer risk (47).

HORMONES

Initial studies decades ago, described the close relationship of testosterone/androgens to prostate growth, and has led to anti-androgen treatment as one of the corner stone of metastatic prostate cancer treatment. However, over the last few years there has been a paradigm shift in our understanding, attitude, and application of testosterone to prostate cancer risk, progression, and survival. The mechanism of androgen receptor saturation model described by Morgentaler *et al.* showed that in the prostate, anything above the baseline serum testosterone concentration will play no further role in stimulating prostate growth, due to the fact that the intraprostatic androgen receptor sites are completely saturated/bound (48).

A recent review by Golla *et al.* analyzed data from studies investigating the use of testosterone in localized prostate cancer on active surveillance, watchful waiting, and definitive treatments. They found that there was no increased risk of prostate cancer diagnosis or more aggressive cancer at diagnosis in men with testosterone supplementation for testosterone deficiency. They also concluded that there is no increased risk of cancer progression in men who are on active surveillance and definitive treatment with testosterone therapy (49).

Regarding 5 alpha reductase inhibitors (5ARIs) and its association with prostate cancer, the two initial studies (PCPT and REDUCE trials) paved way to our understanding of 5ARI in relation to treatment of lower urinary tract symptoms caused by benign prostatic hyperplasia and risk of prostate cancer. 5ARIs prevent the conversion of testosterone to dihydrotestosterone which is the active hormone that regulates growth in prostate cells. In both trials, there was significant risk reduction (22–24%) in developing prostate cancer, but there was increased risk of high-grade prostate cancer at diagnosis (50, 51). However, there were explanations later following further sub analysis that accounted for the increased risk of high-grade disease.

Furthermore, two large studies confirmed the effectiveness of 5ARIs in reducing the risk of developing prostate cancer. Unger *et al.* showed that in 16-year follow-up, there was 21.1% decreased risk of prostate cancer in men who had finasteride compared with placebo and suggested that short term, seven-years, usage of finasteride could provide long term benefit in preventing prostate cancer (52). This finding was further supported by another study in Sweden in which 23,442 men who had treatment with 5ARIs for eight years resulted in reduction in the overall risk of developing prostate cancer, and the effect was larger with longer drug exposure. It also revealed that 5ARI treatment did not affect the long-term risk of developing high-grade cancer at diagnosis (53).

INFECTION, INFLAMMATION AND CHEMOKINES

Chronic inflammation is often a result of numerous exogenous stimuli like infections, radiation, hormones, chemicals, and other noxious stimuli. Following on from this, cancers can often be a subsequent chain of events related chronic inflammation. The key feature of cancer-related inflammation is the recruitment of leukocytes, production of cytokines and chemokines, and subsequent progression, angiogenesis, epithelial-mesenchymal transition (EMT), migration, and metastasis (54). Prostate cancer is no different and numerous studies have investigated the role of chemokines produced by cancer cells and prostate cancer-related chronic inflammation pathway. Chemokines are chemotactic cytokines that influence immune responses and inflammation (54). These inflammatory milieus will then interact with the tumor microenvironment and can lead to development and progression of the tumor. Examples of such important chemokines in prostate cancer are CXCR—upregulated in prostate cancer , and DARC—absence of which will lead to increased incidence and mortality of prostate cancer (16). Better understanding of chemokines and its receptor axis in the tumor microenvironment will pave way for future chemokine targeted therapies in prostate cancer.

CONCLUSION

In summary, although there are many putative risk factors for prostate cancer, apart from ethnicity and age, there is no confirmative etiological factor. Even with age, the frequent use of imaging modalities for other causes resulting in increased incidental finding of prostate cancer in younger men appear to cast doubt on the role of age being a risk factor. While a family history of prostate cancer helps early monitoring of susceptible individuals, more studies need to be done to ascertain the true role of family history in prostate cancer. Results of studies suggesting the role of smoking, alcohol, diet, physical activity, and other non-genetic factors being risk factor for prostate cancer are equivocal. GWAS have great potential, and the identification of SNPs may enable a specific screening program replacing the non-specific PSA screening and avoid unnecessary prostate biopsies.

Conflict of interest: The author declares no potential conflicts of interest with respect to research, authorship and/or publication of this article.

REFERENCES

1. Bray F, Ferlay J, Soerjomataram I, Siegel RL, Torre LA, Jemal A. Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J Clin. 2018;68(6):394–424. https://doi.org/10.3322/caac.21492

2. Ferlay J, Soerjomataram I, Dikshit R, Eser S, Mathers C, Rebelo M, et al. Cancer incidence and mortality worldwide: sources, methods and major patterns in GLOBOCAN 2012. Int J Cancer. 2015;136(5):E359–86. https://doi.org/10.1002/ijc.29210
3. Newcomer LM, Stanford JL, Blumenstein BA, Brawer MK. Temporal trends in rates of prostate cancer: declining incidence of advanced stage disease, 1974 to 1994. J Urol. 1997;158(4):1427–30. https://doi.org/10.1016/S0022-5347(01)64231-9
4. Carter HB, Piantadosi S, Isaacs JT. Clinical evidence for and implications of the multistep development of prostate cancer. J Urol. 1990;143(4):742–6. https://doi.org/10.1016/S0022-5347(17)40078-4
5. Kalish LA, McDougal WS, McKinlay JB. Family history and the risk of prostate cancer. Urology. 2000;56(5):803–6. https://doi.org/10.1016/S0090-4295(00)00780-9
6. Chen YC, Page JH, Chen R, Giovannucci E. Family history of prostate and breast cancer and the risk of prostate cancer in the PSA era. Prostate. 2008;68(14):1582–91. https://doi.org/10.1002/pros.20825
7. Mucci LA, Hjelmborg JB, Harris JR, Czene K, Havelick DJ, Scheike T, et al. Familial Risk and Heritability of Cancer Among Twins in Nordic Countries. JAMA. 2016;315(1):68–76. https://doi.org/10.1001/jama.2015.17703
8. Barber L, Gerke T, Markt SC, Peisch SF, Wilson KM, Ahearn T, et al. Family History of Breast or Prostate Cancer and Prostate Cancer Risk. Clin Cancer Res. 2018;24(23):5910–7. https://doi.org/10.1158/1078-0432.CCR-18-0370
9. Amin Al Olama A, Dadaev T, Hazelett DJ, Li Q, Leongamornlert D, Saunders EJ, et al. Multiple novel prostate cancer susceptibility signals identified by fine-mapping of known risk loci among Europeans. Hum Mol Genet. 2015;24(19):5589–602. https://doi.org/10.1093/hmg/ddv203
10. Hooker S, Hernandez W, Chen H, Robbins C, Torres JB, Ahaghotu C, et al. Replication of prostate cancer risk loci on 8q24, 11q13, 17q12, 19q33, and Xp11 in African Americans. Prostate. 2010;70(3):270–5. https://doi.org/10.1002/pros.21061
11. Benafif S, Kote-Jarai Z, Eeles RA, Consortium P. A Review of Prostate Cancer Genome-Wide Association Studies (GWAS). Cancer Epidemiol Biomarkers Prev. 2018;27(8):845–57. https://doi.org/10.1158/1055-9965.EPI-16-1046
12. Schumacher FR, Al Olama AA, Berndt SI, Benlloch S, Ahmed M, Saunders EJ, et al. Association analyses of more than 140,000 men identify 63 new prostate cancer susceptibility loci. Nat Genet. 2018;50(7):928–36. https://doi.org/10.1038/s41588-018-0142-8
13. Pernar CH, Ebot EM, Wilson KM, Mucci LA. The Epidemiology of Prostate Cancer. Cold Spring Harb Perspect Med. 2018;8(12). https://doi.org/10.1101/cshperspect.a030361
14. Howlader N, Noone A, Krapcho M, Miller D, Bishop K. SEER Cancer Statistics Review 1975–2013. National Cancer Institute, Bethesda MD; 2016.
15. Haiman CA, Chen GK, Blot WJ, Strom SS, Berndt SI, Kittles RA, et al. Characterizing genetic risk at known prostate cancer susceptibility loci in African Americans. PLoS Genet. 2011;7(5):e1001387. https://doi.org/10.1371/journal.pgen.1001387
16. Rani A, Dasgupta P, Murphy JJ. Prostate Cancer: The Role of Inflammation and Chemokines. Am J Pathol. 2019;189(11):2119–37. https://doi.org/10.1016/j.ajpath.2019.07.007
17. Huncharek M, Haddock KS, Reid R, Kupelnick B. Smoking as a risk factor for prostate cancer: a meta-analysis of 24 prospective cohort studies. Am J Public Health. 2010;100(4):693–701. https://doi.org/10.2105/AJPH.2008.150508
18. Zhao J, Stockwell T, Roemer A, Chikritzhs T. Is alcohol consumption a risk factor for prostate cancer? A systematic review and meta-analysis. BMC cancer. 2016;16(1):845. https://doi.org/10.1186/s12885-016-2891-z
19. Ma J, Li H, Giovannucci E, Mucci L, Qiu W, Nguyen PL, et al. Prediagnostic body-mass index, plasma C-peptide concentration, and prostate cancer-specific mortality in men with prostate cancer: a long-term survival analysis. Lancet Oncol. 2008;9(11):1039–47. https://doi.org/10.1016/S1470-2045(08)70235-3
20. Cao Y, Ma J. Body mass index, prostate cancer-specific mortality, and biochemical recurrence: a systematic review and meta-analysis. Cancer Prev Res. 2011;4(4):486–501. https://doi.org/10.1158/1940-6207.CAPR-10-0229

21. Smith LA, O'Flanagan CH, Bowers LW, Allott EH, Hursting SD. Translating Mechanism-Based Strategies to Break the Obesity-Cancer Link: A Narrative Review. J Acad Nutr Diet. 2018;118(4):652–67. https://doi.org/10.1016/j.jand.2017.08.112
22. Saunders TJ, Palombella A, McGuire KA, Janiszewski PM, Després JP, Ross R. Acute exercise increases adiponectin levels in abdominally obese men. J Nutr Metab. 2012;2012:148729. https://doi.org/10.1155/2012/148729
23. Liao Q, Long C, Deng Z, Bi X, Hu J. The role of circulating adiponectin in prostate cancer: a meta-analysis. Int J Biol Markers. 2015;30(1):e22–31. https://doi.org/10.5301/jbm.5000124
24. Gacci M, Russo GI, De Nunzio C, Sebastianelli A, Salvi M, Vignozzi L, et al. Meta-analysis of metabolic syndrome and prostate cancer. Prostate Cancer Prostatic Dis. 2017;20(2):146–55. https://doi.org/10.1038/pcan.2017.1
25. Sourbeer KN, Howard LE, Andriole GL, Moreira DM, Castro-Santamaria R, Freedland SJ, et al. Metabolic syndrome-like components and prostate cancer risk: results from the Reduction by Dutasteride of Prostate Cancer Events (REDUCE) study. BJU Int. 2015;115(5):736–43. https://doi.org/10.1111/bju.12843
26. Kenfield SA, Stampfer MJ, Giovannucci E, Chan JM. Physical activity and survival after prostate cancer diagnosis in the health professionals follow-up study. J Clin Oncol. 2011;29(6):726–32. https://doi.org/10.1200/JCO.2010.31.5226
27. Trudeau K, Rousseau MC, Parent M. Extent of Food Processing and Risk of Prostate Cancer: The PROtEuS Study in Montreal, Canada. Nutrients. 2020;12(3). https://doi.org/10.3390/nu12030637
28. Godos J, Bella F, Sciacca S, Galvano F, Grosso G. Vegetarianism and breast, colorectal and prostate cancer risk: an overview and meta-analysis of cohort studies. J Hum Nutr Diet. 2017;30(3):349–59. https://doi.org/10.1111/jhn.12426
29. Rowles JL, Ranard KM, Smith JW, An R, Erdman JW. Increased dietary and circulating lycopene are associated with reduced prostate cancer risk: a systematic review and meta-analysis. Prostate Cancer Prostatic Dis. 2017;20(4):361–77. https://doi.org/10.1038/pcan.2017.25
30. Applegate CC, Rowles JL, Ranard KM, Jeon S, Erdman JW. Soy Consumption and the Risk of Prostate Cancer: An Updated Systematic Review and Meta-Analysis. Nutrients. 2018;10(1). https://doi.org/10.3390/nu10010040
31. Lippman SM, Klein EA, Goodman PJ, Lucia MS, Thompson IM, Ford LG, et al. Effect of selenium and vitamin E on risk of prostate cancer and other cancers: the Selenium and Vitamin E Cancer Prevention Trial (SELECT). JAMA. 2009;301(1):39–51. https://doi.org/10.1001/jama.2008.864
32. Yarmolinsky J, Bonilla C, Haycock PC, Langdon RJQ, Lotta LA, Langenberg C, et al. Circulating Selenium and Prostate Cancer Risk: A Mendelian Randomization Analysis. J Natl Cancer Inst. 2018;110(9):1035–8. https://doi.org/10.1093/jnci/djy081
33. Park Y, Leitzmann MF, Subar AF, Hollenbeck A, Schatzkin A. Dairy food, calcium, and risk of cancer in the NIH-AARP Diet and Health Study. Arch Intern Med. 2009;169(4):391–401. https://doi.org/10.1001/archinternmed.2008.578
34. Allen NE, Key TJ, Appleby PN, Travis RC, Roddam AW, Tjønneland A, et al. Animal foods, protein, calcium and prostate cancer risk: the European Prospective Investigation into Cancer and Nutrition. Br J Cancer. 2008;98(9):1574–81. https://doi.org/10.1038/sj.bjc.6604331
35. Feldman D, Zhao XY, Krishnan AV. Vitamin D and prostate cancer. Endocrinology. 2000;141(1):5–9. https://doi.org/10.1210/endo.141.1.7341
36. Chan JM, Stampfer MJ, Ma J, Gann P, Gaziano JM, Pollak M, et al. Insulin-like growth factor-I (IGF-I) and IGF binding protein-3 as predictors of advanced-stage prostate cancer. J Natl Cancer Inst. 2002;94(14):1099–106. https://doi.org/10.1093/jnci/94.14.1099
37. Wiseman M. The second World Cancer Research Fund/American Institute for Cancer Research expert report. Food, nutrition, physical activity, and the prevention of cancer: a global perspective. Proc Nutr Soc. 2008;67(3):253–6. https://doi.org/10.1017/S002966510800712X
38. Chan JM, Holick CN, Leitzmann MF, Rimm EB, Willett WC, Stampfer MJ, et al. Diet after diagnosis and the risk of prostate cancer progression, recurrence, and death (United States). Cancer Causes Control. 2006;17(2):199–208. https://doi.org/10.1007/s10552-005-0413-4

39. Richman EL, Stampfer MJ, Paciorek A, Broering JM, Carroll PR, Chan JM. Intakes of meat, fish, poultry, and eggs and risk of prostate cancer progression. Am J Clin Nutr. 2010;91(3):712–21. https://doi.org/10.3945/ajcn.2009.28474
40. Wilson KM, Mucci LA. Diet and Lifestyle in Prostate Cancer. Adv Exp Med Biol. 2019;1210:1–27. https://doi.org/10.1007/978-3-030-32656-2_1
41. Olmedo-Requena R, Lozano-Lorca M, Salcedo-Bellido I, Jiménez-Pacheco A, Vázquez-Alonso F, García-Caballos M, et al. Compliance with the 2018 World Cancer Research Fund/American Institute for Cancer Research Cancer Prevention Recommendations and Prostate Cancer. Nutrients. 2020;12(3).
42. Ahn HK, Lee YH, Koo KC. Current Status and Application of Metformin for Prostate Cancer: A Comprehensive Review. Int J Mol Sci. 2020;21(22). https://doi.org/10.3390/ijms21228540
43. Feng Z, Zhou X, Liu N, Wang J, Chen X, Xu X. Metformin use and prostate cancer risk: A meta-analysis of cohort studies. Medicine (Baltimore). 2019;98(12):e14955. https://doi.org/10.1097/MD.0000000000014955
44. Yu O, Eberg M, Benayoun S, Aprikian A, Batist G, Suissa S, et al. Use of statins and the risk of death in patients with prostate cancer. J Clin Oncol. 2014;32(1):5–11. https://doi.org/10.1200/JCO.2013.49.4757
45. Harshman LC, Wang X, Nakabayashi M, Xie W, Valenca L, Werner L, et al. Statin Use at the Time of Initiation of Androgen Deprivation Therapy and Time to Progression in Patients With Hormone-Sensitive Prostate Cancer. JAMA Oncol. 2015;1(4):495–504. https://doi.org/10.1001/jamaoncol.2015.0829
46. Jian Z, Ye D, Chen Y, Li H, Wang K. Sexual Activity and Risk of Prostate Cancer: A Dose-Response Meta-Analysis. J Sex Med. 2018;15(9):1300–9. https://doi.org/10.1016/j.jsxm.2018.07.004
47. Bhindi B, Wallis CJD, Nayan M, Farrell AM, Trost LW, Hamilton RJ, et al. The Association Between Vasectomy and Prostate Cancer: A Systematic Review and Meta-analysis. JAMA Intern Med. 2017;177(9):1273–86. https://doi.org/10.1001/jamainternmed.2017.2791
48. Morgentaler A, Traish AM. Shifting the paradigm of testosterone and prostate cancer: the saturation model and the limits of androgen-dependent growth. Eur Urol. 2009;55(2):310–20. https://doi.org/10.1016/j.eururo.2008.09.024
49. Golla V, Kaplan AL. Testosterone Therapy on Active Surveillance and Following Definitive Treatment for Prostate Cancer. Curr Urol Rep. 2017;18(7):49. https://doi.org/10.1007/s11934-017-0695-6
50. Thompson IM, Goodman PJ, Tangen CM, Lucia MS, Miller GJ, Ford LG, et al. The influence of finasteride on the development of prostate cancer. N Engl J Med. 2003;349(3):215–24. https://doi.org/10.1056/NEJMoa030660
51. Andriole GL, Bostwick DG, Brawley OW, Gomella LG, Marberger M, Montorsi F, et al. Effect of dutasteride on the risk of prostate cancer. N Engl J Med. 2010;362(13):1192–202. https://doi.org/10.1056/NEJMoa0908127
52. Unger JM, Hershman DL, Till C, Tangen CM, Barlow WE, Ramsey SD, et al. Using Medicare Claims to Examine Long-term Prostate Cancer Risk of Finasteride in the Prostate Cancer Prevention Trial. J Natl Cancer Inst. 2018;110(11):1208–15. https://doi.org/10.1093/jnci/djy035
53. Wallerstedt A, Strom P, Gronberg H, Nordstrom T, Eklund M. Risk of Prostate Cancer in Men Treated With 5α-Reductase Inhibitors-A Large Population-Based Prospective Study. J Natl Cancer Inst. 2018;110(11):1216–21. https://doi.org/10.1093/jnci/djy036
54. Hughes CE, Nibbs RJB. A guide to chemokines and their receptors. FEBS J. 2018;285(16):2944–71. https://doi.org/10.1111/febs.14466

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The Pathogenesis of Prostate Cancer

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Doi: https://doi.org/10.36255/exonpublications.prostatecancer.pathogenesis.2021

Abstract: Prostate cancer is a major cause of pathology in men world-wide and is age-related. Rare in the under 40s, a third of all those over 80 have been shown to have prostate lesions at autopsy. Both hereditary and molecular influences appear to be involved in the pathogenesis of the condition. Androgenic receptors play a major role in most, but not all, prostate cancers. The cell type involved is related to the aggressiveness of the malignancy. Of those that develop the disease, some die with prostate cancer, others because of it. Over 90% of the cancers are adenocarcinomas. The likelihood of progression of the disease can, but only to a degree, be predicted on histological examination, according to the Gleason Scale and its modifications. These assess degrees of tissue differentiation. Use of blood levels of prostate specific antigen levels as an indication of the activity of tumors is also not straightforward. Our understanding of the disease mechanisms needs further expansion if we are to advance diagnosis of aggressive tumors and develop more effective therapies.

Keywords: androgenic regulation; neuroendocrine cells; prostate cancer; prostatic intraepithelial neoplasia; transrectal ultrasound

In: *Prostate Cancer*. Bott SRJ, Ng KL (Editors). Exon Publications, Brisbane, Australia. ISBN: 978-0-6450017-5-4; Doi: https://doi.org/10.36255/exonpublications.prostatecancer.2021

INTRODUCTION

In the United Kingdom (UK), prostate cancer is the most prevalent neoplasm in males, accounting for 13% of total cancer cases (1). Prostate cancer contributes to 7% of all cancer-related deaths, making it the second most common cause of cancer death in males. Age-standardized incidence rates for prostate cancer have increased by 41% since 1993 although mortality rates have dropped 10% in the last decade (1). It is a heterogenous disease, with a wide scope of disease pathogenesis from asymptomatic and prolonged, where men die with the disease, to severe malignancy where men die from it or have significant morbidity. At the turn of the century, a Spanish study evaluating prostate histology at autopsy identified that men from the third decade onwards have neoplastic lesions of the prostate and 33% of the male population in their eighth decade have prostate cancer (2). In addition, prostate cancer is the most commonly diagnosed malignancy in older men (over the age of 65) worldwide, especially in highly developed countries (Australia, New Zealand, Northern and Western Europe, and North America) (3–5). The high prevalence of the disease can be attributed to the volume of cases identified through prostate specific antigen (PSA) testing, especially in the United States (6). However, prostate cancer screening has been surrounded by controversy and can be seen as an ethical dilemma. Studies have shown that distinguishing between indolent pathology and aggressive tumors using PSA levels alone is insufficient as it is not cancer-specific (7). It is also a challenge as high PSA levels do not directly correlate with pathogenicity and requires further investigations through multiparametric magnetic resonance imaging (MRI) and histology. Therefore, early diagnosis often leads to harm to patients via overdiagnosis and unnecessary treatment (radical surgery, radiotherapy and chemical castration) leading to reduced quality of life (QoL) and psychological trauma knowing they have a cancer diagnosis (7). This chapter aims to address the developmental processes involved in prostate cancer including hereditary and molecular components.

PROSTATE CARCINOGENESIS

The prostate is a male sex glandular structure, derived embryologically from the urogenital sinus. The glandular tissue consists of multiple secretory units (acini), consisting of ducts lined with epithelial cells, that converge and open either side of the verumontanum. Its main functions are to provide force to ejaculate semen and to add nutrient-rich alkaline fluid to the semen to maintain spermatic health post-ejaculation and enhance fertility (8). The gland is highly susceptible to malignant transformation and as a consequence has a higher rate of malignancy than other structures in the urogenital tract. Studies have shown that the characterization of prostatic carcinogenesis is closely linked to organogenesis embryologically, including a heavy reliance on androgenic hormone signaling, such as testosterone, as well as debated potential links to other embryological signaling pathways such as Sonic Hedgehog expression (Shh) and inappropriate expression of the Gli-1 oncogene leading to stromal tumor growth and proliferation (9–11).

HISTOPATHOLOGY OF PROSTATE CANCER

Prostate cancer involves malignancy primarily of the epithelium and is thus classed as a carcinoma. There are rarer subtypes of prostate cancer such as sarcomas (derived from mesenchyme) and lymphomas (12). Neoplastic changes normally arise in the peripheral glandular tissue of the prostate. The prostatic epithelium comprises of luminal, basal and rare neuroendocrine (NE) cell types (13). The epithelial luminal cells, expressing androgen receptors (ARs), cover the internal surface of the prostatic ducts and secrete prostatic fluid and the glycoprotein PSA. They are surrounded by basal cells, that produce proteins used for fluid production and the formation of the acinar basement membrane that separates the epithelial acini from the prostatic stroma. These cells have interspersed NE cells. Both basal and NE cell types are deficient in ARs and thus are not testosterone or androstenedione dependent (14). Fibroblasts, smooth muscle and infiltrating immunological cells combine together to form the stroma of the prostate. There is currently a large interest in identifying the cell type that is responsible for oncogenic transformation (cell of origin) in prostate cancer due to the variability of disease progression and the unpredictability of treatment response (13, 15). Prostate cancer biopsies show tissue deficient in basal cells, leading to questions as to whether there is a form of basal cell differentiation into luminal cells or that luminal cells are the primary cell of origin (13). Using these cell types, it has been hypothesized that tumors arising from luminal cells will be more aggressive than those arising from basal cells (16). About 90–95% of prostate cancers are acinar adenocarcinomas that arise from the peripheral prostatic gland (17). Histological diagnosis is made by assessing the loss of surrounding basal cells, loss of normal glandular architecture, including the disruption to the epithelial-stromal basement membrane, and nuclear atypia of luminal cells (Figure 1) (18). Aggressiveness of the adenocarcinoma is reflected in the degree of differentiation on histology. This is graded using a Gleason Score grading system, last modified in 2014 by the International Society of Urologic Pathology (ISUP) (19). This stratifies histological findings of prostate cancer with prognostic behavior, that is, the 5-year biochemical recurrence (BCR) risk following radical prostatectomy (Table 1) (20). Prostate cancer is staged using the 2018 classification for adenocarcinoma of the prostate based on primary tumor (T), lymph node involvement (N) and metastases (M). Prostate cancer typically involves regional lymph nodes in the pelvis below the bifurcation of the common iliac arteries, and metastases that are outside of the true pelvis, most commonly bone and in advanced disease, lung, and liver (21).

PRECURSOR OF PROSTATE CANCER

Prostate cancer involves the transformation of benign epithelial cells into their malignant phenotype. The most frequent process of cancer transformation in the prostate is called prostatic intraepithelial neoplasia (PIN) (22). PIN is a multicentric condition and is defined as the “neoplastic growth within the pre-existing benign epithelium of the acini or ducts” (23). PIN can be divided into two grades,

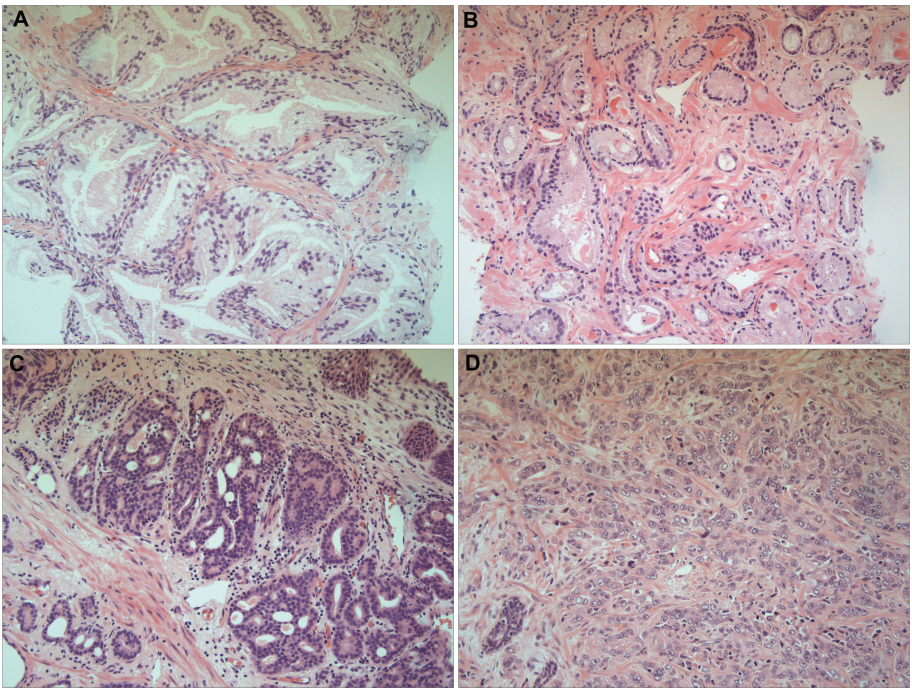

Figure 1. Prostate histology in Gleason grading groups. Gleason scoring allows physicians to predict the prognosis of patients by assessing the histological patterns of their disease. **A.** Normal prostate tissue showing well-formed and well demarcated glands. **B.** Grade Score 3 prostatic glands are well-formed and individualized meaning they are well delineated with a clear stroma. **C.** Gleason Score 4 glands become fused together, are poorly formed, and show a cribriform pattern. **D.** Gleason Score 5 there is a lack of gland formation and the presence of individual cells. This particular section also demonstrates cytoplasmic vacuoles. (Images kindly provided by Dr. Maria Bahhadi-Hardo, Consultant Histopathologist, Frimley Park Hospital, Frimley Surrey, UK).

low (LGPIN) and high (HGPIN). HGPIN has a high predictive value for predicting progression to adenocarcinoma. HGPIN can only be detected on needle biopsy, it does not raise serum PSA levels and is not identified on transrectal ultrasound (TRUS) (24, 25). Histologically there are four main findings: micropapillary, tufting, cribriform and flat (25). These findings are of diagnostic value only and their individual presence/absence does not predict tumor aggressiveness (25). The basal cell layer is mostly intact in HGPIN with minimal stromal invasion. HGPIN is clinically significant, with patients requiring repeat biopsy as surveillance, with a recommended interval of 3–6 months for 2 years then yearly for life (26). Multiple studies have reported HGPIN as a significant predictor for occurrence of prostate cancer (22–58%), therefore the presence of HGPIN on biopsy may warrant therapeutic treatment in the future (25).

TABLE 1 **Gleason grading groups (19, 20)**

Grade group 1: Gleason score ≤6
Only individual discrete well-formed glands
96% 5-year BCR free progression

Grade group 2: Gleason score 3+4 = 7
Predominantly well-formed glands with lesser component of poorly formed/fused/cribriform glands
88% 5-year BCR free progression

Grade group 3: Gleason score 4+3 = 7
Predominantly poorly formed/fused/cribriform glands with lesser component of well-formed glands
63% 5-year BCR free progression

Grade group 4: Gleason score 4+4 = 8; 3+5 = 8; or 5+3 = 8
Only poorly formed/fused/cribriform glands, OR Predominantly well-formed glands and lesser component lacking glands, or Predominantly lacking glands and lesser component of well-formed glands
48% 5-year BCR free progression

Grade group 5: Gleason scores 9–10
Lack gland formation (or with necrosis) with or without poorly formed/fused/cribriform glands
26% 5-year BCR free progression

ANDROGENIC REGULATION OF PROSTATE CANCER

ARs are essential transcription factors in the regulation of male sexual development and accessory sexual organ maintenance (27). Prostate cancer growth and disease progression is initially dependent on AR activation, *via* testosterone and dihydrotestosterone (DHT), leading to nuclear translocation of the receptor and subsequent binding to androgen response elements (AREs) initiating transcription of genes that regulate cellular differentiation, proliferation and apoptosis (28) (Figure 2). Therefore, AR stimulation is integral to the maintenance and survival of prostate luminal epithelial cells. The hypothalamic-pituitary-gonadal (HPG) axis regulates the production of androgens. Androgens are primarily produced in Leydig cells of the testes under the regulation of luteinizing hormone (LH) secreted by the anterior pituitary gland, which in turn is regulated by gonadotropin-releasing hormone (GnRH). Testosterone is converted into DHT in the epithelial cells of the prostate by 5α-reductase which acts as a potent ligand to cytoplasmic ARs.

Studies have failed to establish a link between raised serum levels of androgens and prostate cancer (29). In fact, high androgen levels are linked to reduced risk of aggressive prostate cancer, whilst patients with low serum androgen levels have higher risk of prostate cancer recurrence and advanced pathology (30). In low-androgen environments, there is a selective pressure for luminal cells to become androgen-independent in order to survive. Therefore, ARs are important clinically as they are seen as integral to the progression of disease in prostate cancer. Due to its role in the development, maintenance and secretory functions of the prostate, the HPG axis is a therapeutic target in the treatment of prostate cancer (31).

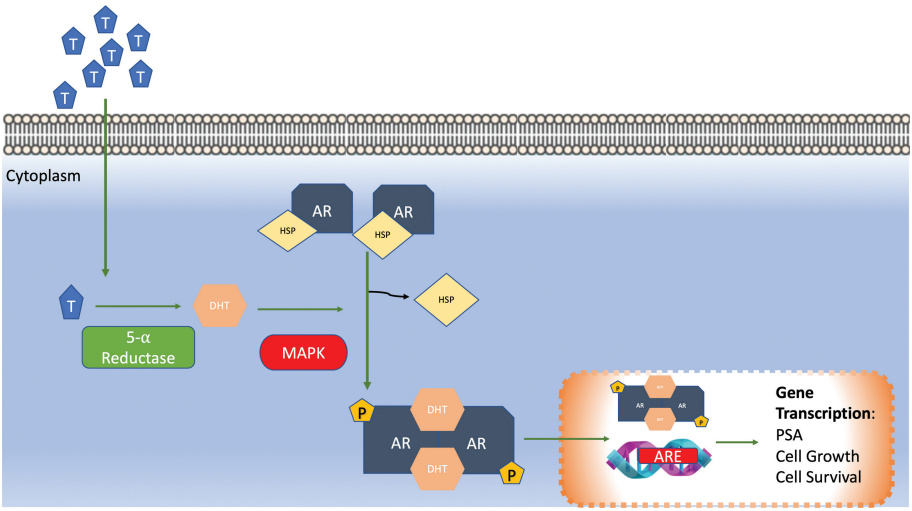

Figure 2. Androgenic regulation of prostate cancer. In the cytoplasm, activity of ARs is regulated by ligand-binding and heat shock proteins (HSP). Testosterone (T) is transported into the cytoplasm of androgen-receptive cells and is converted to DHT by 5-α reductase. DHT ligand binding leads to dissociation from HSP, MAPK then phosphorylates the receptor, this is followed by dimerization. The AR dimer then translocates into the nucleus where it binds to androgen response elements (AREs) in the DNA activating transcription of elements that are essential for cell growth and survival.

ARs have four functional domains: an amino-terminal transcription activation domain (NTD), a DNA-binding domain (DBD) that fastens the AR to the ARE of AR-regulated genes, a hinge region, and a C-terminal ligand-binding domain (LBD) that contains a ligand-binding pocket (LBP) that binds to androgens. When a ligand is bound, the LBD undergoes a conformational change allowing it to recruit co-factors, recognize the DNA sequences, along with the NTD, and initiate the transcription of these genes (such as *PSA*) and facilitating male sexual development (32). ARs have two active functional domains (AFs) that initiate transcription when activated (33). AF-1 is present in the NTD and its activation is androgen independent. AF-2 is located in the LBD and is androgen ligand-dependent (34). The LBP of the LBD is a therapeutic target and is targeted with treatments such as bicalutamide. AF-1 may enable cross-coupling between androgenic and growth factor signaling pathways (33). Therefore, these AFs are deemed clinically important as they could provide the key to understanding castration-resistant prostate cancer (CRPC).

THEORETICAL PATHWAYS IN THE PROGRESSION TO CASTRATION-RESISTANT PROSTATE CANCER

Androgenic blockade, through LBP and 5α-reductase antagonists, as well as HPG overstimulation *via* LH/GnRH analogues, leads to epithelial cell apoptosis and a

transient response in preventing proliferation in prostate cancer treatment. Patients will inevitably develop CRPC and thus develop a more lethal form of prostate cancer. However, the distinct pathological processes involved in this transformation is yet to be fully described as it is likely to involve a multitude of mechanisms. Studies have reported *AR* mutations play an important role in the malignant potential of CRPC and that androgen-independent prostate cancer cells exhibit high levels of *AR* genetic amplification (35). *AR* mutations in primary prostate cancer are rare, however these mutations are prevalent in CRPC (50%) (36, 37). The *AR* gene mutations database describes 1029 associated *AR* mutations, of which 159 are related to predisposing men to prostate cancer (38). These mutations lead to alterations that improve the functional activity of the receptor, such as increased AR sensitivity to low levels of ligand (35, 39), non-androgen ligand binding, ligand-independent activation as well as AR-independent pathways (32). Further studies and meta-analyses have identified that shortened *AR* CAG-repeats in the NTD may increase the risk of prostate cancer (32) and the genetic links are further supported by familial risk, with men having 2–4 times risk if a first-degree relative has prostate cancer (40).

ANDROGEN RECEPTOR 'CROSS-TALK' WITH PEPTIDE GROWTH FACTORS

AR-independent pathways include alternative peptide growth factors including transforming growth factor-β (TGF-β), epidermal growth factor (EGF), fibroblast growth factor (FGF) and insulin-like growth factor (IGF) (41, 42). These peptide factors help facilitate the AR-regulated proliferation of prostate epithelial cells *via* a process called 'cross-talk' (42). For instance, EGF, alongside its membrane-associated tyrosine receptor kinase EGF-1 (EGFR, HER1), is involved in the motility and invasiveness of cancer cells through enhanced migration through extracellular matrix barriers, basal membranes and then the subsequent cellular proliferation (43). ErbB2 (HER2), a member of the EGFR family, is upregulated in CRPC and has been associated with androgen-independent transcriptional activation of ARs and the subsequent heightened expression of PSA (29). HER2/neu receptors, part of the EGFR family, are found overexpressed in breast and ovarian cancers. They have been proposed as a mechanism for AR-independent activation in CRPC (44). *In vitro* and *in vivo* studies involving forced overexpression of HER2/neu receptors or transfection of HER2/neu in prostate cancer cell lines (LNCaP) identified promotion to AR-independence and expression of PSA (44, 45).

ANDROGEN RECEPTOR BYPASS PATHWAYS

The reduction in AR activation leads to hypersensitization of other pathways. For instance, the upregulation in IGF-1, EGF and other growth factors subsequently activate ErbB2 and other tyrosine receptor kinases (32). This results in the activation of phosphatidylinositol 3-kinase (PI3K) and subsequent PI3K-AKT-mTOR pathway (46, 47). PI3K activation, converts phosphatidylinositol

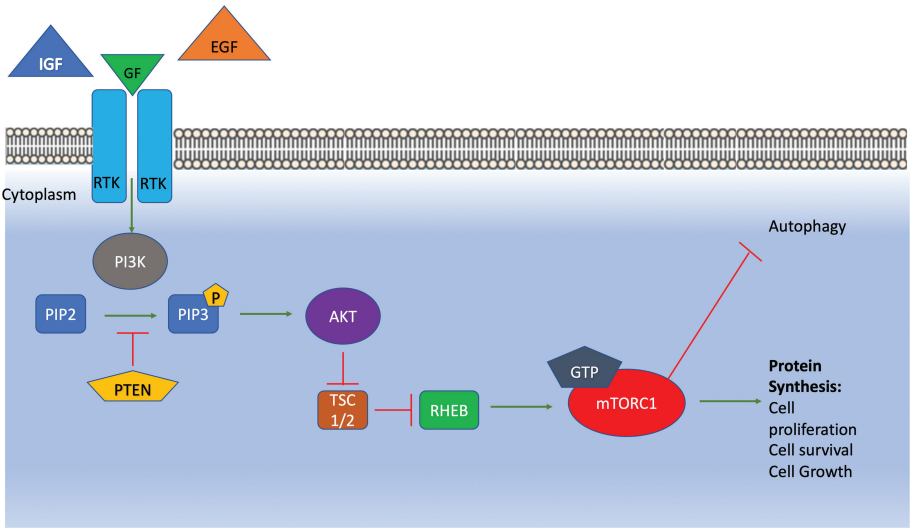

Figure 3. Androgen receptor bypass pathways. Reduced AR suppression leads to the upregulation of Tyrosine Receptor Kinases (RTK) e.g., ErbB2 by factors such as IGF, GF and EGF. RTK activation leads to the stimulation of PI3K with phosphorylates PIP2 into PIP3. This process is inhibited by the tumor suppressor PTEN. PIP3 activates AKT which indirectly suppresses the activity of the cell survival protein mTORC1 by inhibiting TSC1/2 which in turn suppresses the GTP-binding protein RHEB. mTORC1 is pivotal in the translation of proteins and therefore protein synthesis. Therefore, AR inhibition, as well as PTEN suppression, leads to overactivation of the PIP3-AKT-mTOR pathway – creating an alternate route for cell survival in CRPC.

4,5-bisphosphonate (PIP2) to phosphatidylinositol 3–5-triphosphate (PIP3) which recruits protein kinase B (AKT) proteins to the luminal cell cytoplasm (46). AKT signaling involves deregulating the tuberous sclerosis 1/2 (TSC1/2), an inhibitor of the GTP-binding protein RHEB, which in turn is responsible for the activation of the mechanistic target of rapamycin complex 1 (mTORC1), a kinase that is critical to the regulation of cell cycle. mTORC1 impedes autophagy and promoting prostate cancer cellular proliferation (Figure 3) (46). Through this pathway, studies have proven that IGF-1 can activate AR-mediated gene transcription and stimulate the production of PSA in LNCaP cells. As a result, the PI3K-AKT-mTOR pathway is of interest in establishing new therapeutic targets for patients with CRPC (47, 48).

THE ROLE OF TUMOR SUPPRESSOR GENE PTEN IN PROSTATE CANCER

Genetic analysis of CRPC tumors has identified that the gene for the protein phosphatase and tensin homolog (*PTEN)* is mutated in 20% of cases (49). *PTEN* is a tumor suppressor gene, it acts by negatively regulating the PI3K-AKT-mTOR pathway and halting the cell-cycle at the G_1 stage therefore halting cellular

proliferation. Loss of PTEN thus results in an increase in the PI3K-AKT-mTOR pathway as well as impairing normal AR regulation, resulting in increased cellular proliferation, AR expression and reduced apoptosis (29). *PTEN's* expression is inversely correlated with Gleason Score and therefore is associated significantly with aggressive prostate cancer (Gleason $\geq$7, P = 0.0004), with up to 20% of high-grade tumors being negative for *PTEN* expression (50).

TUMOR MICROENVIRONMENT IN PROSTATE CANCER

The tumor microenvironment (TME) describes the vast array of supporting cells (including immune cells, fibroblasts, adipose cells, microvasculature, and components of the extracellular matrix [ECM]) that form a complex network surrounding tumor cells that may play a role in their pathogenicity, especially involving their transition from normal cells to neoplastic cancer cells themselves (51, 52). Studies have reported that tumor cells are able to 'hijack' immune cells and prime them to aid metastatic potential (51). As studied by Chen et al. (53), immune cells can be infiltrated by tumor cells forming tumor-associated macrophages (TAMs). They identified that CD8-T cells can express PSA in aggressive forms of prostate cancer, and this perhaps enhances the tumor's ability to metastasize to lymphatic tissue and bone. Therefore, the TME may provide the environment whereby alternative cell types transform into malignant tumor cells in aggressive prostate cancer and influence the ability to invade local and systemic structures (54).

APOPTOSIS REGULATION

Dysregulation in the programmed cell death mechanisms (apoptosis) is important in the pathogenesis of prostate cancer and is deemed a key driver in the exponential growth of tumor cells (55). There are two distinct pathways, intrinsic and extrinsic, that are involved in the normal signaling of programmed cell death that are critical to tissue homeostasis. Extrinsic (receptor pathway) mechanisms involve the intracellular binding of apoptosis-inducing ligands, such as tumor necrosis factor (TNF), to cell-surface death receptors that are part of the TNF-R superfamily such as CD95 (Apo-1/Fas) or TNF-related apoptosis-inducing ligand (TRAIL) receptors. These receptors contain the Fas-associated death domain (FADD) and form death-inducing signaling complexes (DISCs) with intracellular caspases that contain a death-effector domain (DED) such as caspases 8 and 10 (56, 57). Effector caspases, including caspase-3, activate proteolysis and cleavage of intracellular/intranuclear substrates, inducing apoptosis (56).

Intrinsic (mitochondrial pathway) mechanisms involve the activation of mitochondria through intracellular damage such as DNA damage *via* chemoradiotherapy. Intracellular insults result in the p53 activation of the pro-apoptotic B-cell lymphoma 2 (Bcl-2) family (including Bax, Bid and Bad) that induce mitochondrial release of cytochrome *c* (Cyc-*c*) (58). Cyc-*c* is a key component, alongside procaspase 9 and apoptotic protease activating factor-1 (Apaf-1), that form an apoptosome, an apoptosis inducing complex (59). The apoptosome then converges alongside the extrinsic pathway in activating caspase-3.

Bcl-2 family members such as Bax, Bid and Bad are proapoptotic factors that target the mitochondrial membrane and facilitate Cyc-*c* release. However, Bcl-2 is permanently anchored to the mitochondrial wall, is anti-apoptotic and prevent the release of Cyc-*c* (55). Therefore, the Bcl-2 family work in homeostasis to regulate cellular death. In aggressive prostate cancer, Bcl-2 is upregulated, swinging the homeostatic balance firmly towards anti-apoptosis (60). This overexpression is seen in chemoradiotherapy-resistant prostate cancer phenotypes, therefore can be seen as a key indicator in the prognosis of aggressive prostate cancer (55, 60). Upstream of Bcl-2 family receptors, mutations of the *p53* tumor suppressor gene are also characteristic of malignant prostate cancer and CRPC, further highlighting the critical role of apoptosis in pathogenicity (61). The true genetic and molecular pathophysiology of prostate cancer is a complex topic. Unlike breast cancer, there are no highly penetrant and dominating genetic mutations that account for the majority of prostate cancer cases. Thus, there is huge scope for future therapeutic innovation and bespoke genetic analysis may well play a role in reducing the prevalence of CRPC.

CONCLUSION

Prostate cancer is a multi-factorial disease entity that is still incompletely understood. A major problem is the current difficulty in assessing risk of progression of the disease which can have a relatively benign course or be rapidly spreading to other tissues with fatal consequences. Expansion of our knowledge of the pathogenesis of prostate cancer is essential if our search for truly predictive markers, and tissue assessments, is to lead to a rapid understanding of potential clinical outcome of a tumor of the prostate, and for the development and use of highly effective treatments that do not lead to some patients being negatively affected by unnecessary procedures and diagnoses.

Conflict of interest: The author declares no potential conflicts of interest with respect to research, authorship and/or publication of this article.

REFERENCES

1. UK CR. Prostate Cancer Mortality Statistics 2021 [Available from: https://www.cancerresearchuk.org/health-professional/cancer-statistics/statistics-by-cancer-type/prostate-cancer/mortality [Accessed on 10 April 2021]
2. Sánchez-Chapado M, Olmedilla G, Cabeza M, Donat E, Ruiz A. Prevalence of prostate cancer and prostatic intraepithelial neoplasia in Caucasian Mediterranean males: an autopsy study. Prostate. 2003;54(3):238–47. https://doi.org/10.1002/pros.10177

3. Brawley OW. Prostate cancer epidemiology in the United States. World J Urol 2012;30(2):195–200. https://doi.org/10.1007/s00345-012-0824-2
4. Mishra SC. A discussion on controversies and ethical dilemmas in prostate cancer screening. J Med Ethics. 2021;47(3):152–8. https://doi.org/10.1136/medethics-2019-105979
5. Bray F, Ferlay J, Soerjomataram I, Siegel RL, Torre LA, Jemal A. Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA: A Cancer J Clin. 2018;68(6):394–424. https://doi.org/10.3322/caac.21492
6. Barry MJ. Screening for prostate cancer--the controversy that refuses to die. N Engl J Med. 2009;360(13):1351. https://doi.org/10.1056/NEJMe0901166
7. Gentile F, Ferro M, Della Ventura B, La Civita E, Liotti A, Cennamo M, et al. Optimized Identification of High-Grade Prostate Cancer by Combining Different PSA Molecular Forms and PSA Density in a Deep Learning Model. Diagnostics. 2021;11(2):335. https://doi.org/10.3390/diagnostics11020335
8. Toivanen R, Shen MM. Prostate organogenesis: tissue induction, hormonal regulation and cell type specification. Development. 2017;144(8):1382–98. https://doi.org/10.1242/dev.148270
9. Karhadkar SS, Steven Bova G, Abdallah N, Dhara S, Gardner D, Maitra A, et al. Hedgehog signalling in prostate regeneration, neoplasia and metastasis. Nature. 2004;431(7009):707–12. https://doi.org/10.1038/nature02962
10. Fan L, Pepicelli CV, Dibble CC, Catbagan W, Zarycki JL, Laciak R, et al. Hedgehog Signaling Promotes Prostate Xenograft Tumor Growth. Endocrinology. 2004;145(8):3961–70. https://doi.org/10.1210/en.2004-0079
11. Shaw A, Gipp J, Bushman W. The Sonic Hedgehog pathway stimulates prostate tumor growth by paracrine signaling and recapitulates embryonic gene expression in tumor myofibroblasts. Oncogene. 2009;28(50):4480–90. https://doi.org/10.1038/onc.2009.294
12. Humphrey PA. Histopathology of prostate cancer. Cold Spring Harb Perspect Med. 2017;7(10):a030411. https://doi.org/10.1101/cshperspect.a030411
13. Lee SH, Shen MM. Cell types of origin for prostate cancer. Curr Opin Cell Biol. 2015;37:35–41. https://doi.org/10.1016/j.ceb.2015.10.002
14. Blagoev KB, Iordanov R, Zhou M, Fojo T, Bates SE. Drug resistant cells with very large proliferative potential grow exponentially in metastatic prostate cancer. Oncotarget. 2020;12(1). https://doi.org/10.18632/oncotarget.27855
15. Wang Z, Shen M. Revisiting the concept of cancer stem cells in prostate cancer. Oncogene. 2011;30(11):1261–71. https://doi.org/10.1038/onc.2010.530
16. Wang ZA, Mitrofanova A, Bergren SK, Abate-Shen C, Cardiff RD, Califano A, et al. Lineage analysis of basal epithelial cells reveals their unexpected plasticity and supports a cell-of-origin model for prostate cancer heterogeneity. Nat Cell Biol. 2013;15(3):274–83. https://doi.org/10.1038/ncb2697
17. Alizadeh M, Alizadeh S. Survey of clinical and pathological characteristics and outcomes of patients with prostate cancer. Glob J Health Sci. 2014;6(7):49. https://doi.org/10.5539/gjhs.v6n7p49
18. Humphrey PA, Moch H, Cubilla AL, Ulbright TM, Reuter VE. The 2016 WHO Classification of Tumours of the Urinary System and Male Genital Organs-Part B: Prostate and Bladder Tumours. Eur Urol. 2016;70(1):106–19. https://doi.org/10.1016/j.eururo.2016.02.028
19. Epstein JI, Egevad L, Amin MB, Delahunt B, Srigley JR, Humphrey PA. The 2014 International Society of Urological Pathology (ISUP) Consensus Conference on Gleason Grading of Prostatic Carcinoma: Definition of Grading Patterns and Proposal for a New Grading System. Am J Surg Pathol. 2016;40(2):244–52. https://doi.org/10.1097/PAS.0000000000000530
20. Barakzai MA. Prostatic Adenocarcinoma: A Grading from Gleason to the New Grade-Group System: A Historical and Critical Review. Asian Pac J Cancer Prev. 2019;20(3):661–6. https://doi.org/10.31557/APJCP.2019.20.3.661
21. Buyyounouski MK, Choyke PL, McKenney JK, Sartor O, Sandler HM, Amin MB, et al. Prostate cancer - major changes in the American Joint Committee on Cancer eighth edition cancer staging manual. CA: Cancer J Clin 2017;67(3):245–53. https://doi.org/10.3322/caac.21391
22. Brawer MK. Prostatic intraepithelial neoplasia: an overview. Rev Urol. 2005;7 Suppl 3(Suppl 3): S11-S8.

23. Kim HL, Yang XJ. Prevalence of high-grade prostatic intraepithelial neoplasia and its relationship to serum prostate specific antigen. Int Braz J Urol. 2002;28(5):413–6.
24. Alexander EE, Qian J, Wollan PC, Myers RP, Bostwick DG. Prostatic intraepithelial neoplasia does not appear to raise serum prostate-specific antigen concentration. Urology. 1996;47(5):693–8. https://doi.org/10.1016/S0090-4295(96)00004-0
25. Bostwick DG, Qian J. High-grade prostatic intraepithelial neoplasia. Mod Pathol. 2004;17(3):360–79. https://doi.org/10.1038/modpathol.3800053
26. Bostwick DG. Prostatic intraepithelial neoplasia (PIN): current concepts. J Cell Biochem. 1992;50(S16H):10–9. https://doi.org/10.1002/jcb.240501205
27. Culig Z, Klocker H, Bartsch G, Hobisch A. Androgen receptors in prostate cancer. Endocr-Relat Cancer. 2002;9(3):155–70. https://doi.org/10.1677/erc.0.0090155
28. Hodges C. Studies on prostatic cancer I. the effect of castration, of estrogen and of androgen injection on serum phosphatases in metastatic carcinoma of the prostate. Cancer Res. 1941;1(4):293–7.
29. Heinlein CA, Chang C. Androgen receptor in prostate cancer. Endocr Rev. 2004;25(2):276–308. https://doi.org/10.1210/er.2002-0032
30. Corona G, Baldi E, Maggi M. Androgen regulation of prostate cancer: where are we now? J Endocrinol Investig. 2011;34(3):232–43. https://doi.org/10.1007/BF03347072
31. Schleutker J. Polymorphisms in androgen signaling pathway predisposing to prostate cancer. Mol Cell Endocrinol. 2012;360(1):25–37. https://doi.org/10.1016/j.mce.2011.07.007
32. Tan MHE, Li J, Xu HE, Melcher K, Yong E-l. Androgen receptor: structure, role in prostate cancer and drug discovery. Acta Pharmacol Sin. 2015;36(1):3–23. https://doi.org/10.1038/aps.2014.18
33. Bevan C, Parker M. The Role of Coactivators in Steroid Hormone Action. Exp Cell Res. 1999;253(2):349–56. https://doi.org/10.1006/excr.1999.4719
34. Girling JS, Whitaker HC, Mills IG, Neal DE. Pathogenesis of prostate cancer and hormone refractory prostate cancer. Indian J Urol. 2007;23(1):35–42. https://doi.org/10.4103/0970-1591.30265
35. Taplin M-E, Bubley GJ, Shuster TD, Frantz ME, Spooner AE, Ogata GK, et al. Mutation of the androgen-receptor gene in metastatic androgen-independent prostate cancer. N Engl J Med. 1995;332(21):1393–8. https://doi.org/10.1056/NEJM199505253322101
36. Chen CD, Welsbie DS, Tran C, Baek SH, Chen R, Vessella R, et al. Molecular determinants of resistance to antiandrogen therapy. Nat Med. 2004;10(1):33–9. https://doi.org/10.1038/nm972
37. Visakorpi T, Hyytinen E, Koivisto P, Tanner M, Keinänen R, Palmberg C, et al. In vivo amplification of the androgen receptor gene and progression of human prostate cancer. Nat Genet. 1995;9(4):401–6. https://doi.org/10.1038/ng0495-401
38. Gottlieb B, Beitel LK, Nadarajah A, Paliouras M, Trifiro M. The androgen receptor gene mutations database: 2012 update. Hum Mutat. 2012;33(5):887–94. https://doi.org/10.1002/humu.22046
39. Koivisto P, Kononen J, Palmberg C, Tammela T, Hyytinen E, Isola J, et al. Androgen receptor gene amplification: a possible molecular mechanism for androgen deprivation therapy failure in prostate cancer. Cancer Res. 1997;57(2):314–9.
40. Stanford JL, Ostrander EA. Familial prostate cancer. Epidemiol Rev. 2001;23(1):19–23. https://doi.org/10.1093/oxfordjournals.epirev.a000789
41. Velcheti V, Karnik S, Bardot SF, Prakash O. Pathogenesis of prostate cancer: lessons from basic research. Ochsner J. 2008;8(4):213–8.
42. Zhu ML, Kyprianou N. Androgen receptor and growth factor signaling cross-talk in prostate cancer cells. Endocr Relat Cancer. 2008;15(4):841–9. https://doi.org/10.1677/ERC-08-0084
43. Wells A, Kassis J, Solava J, Turner T, Lauffenburger DA. Growth Factor-Induced Cell Motility in Tumor Invasion. Acta Oncol. 2002;41(2):124–30. https://doi.org/10.1080/028418602753669481
44. Craft N, Shostak Y, Carey M, Sawyers CL. A mechanism for hormone-independent prostate cancer through modulation of androgen receptor signaling by the HER-2/neu tyrosine kinase. Nat Med. 1999;5(3):280–5. https://doi.org/10.1038/6495
45. Yeh S, Lin HK, Kang HY, Thin TH, Lin MF, Chang C. From HER2/Neu signal cascade to androgen receptor and its coactivators: a novel pathway by induction of androgen target genes through MAP kinase in prostate cancer cells. Proc Natl Acad Sci U S A. 1999;96(10):5458–63. https://doi.org/10.1073/pnas.96.10.5458

46. Sarker D, Reid AHM, Yap TA, de Bono JS. Targeting the PI3K/AKT Pathway for the Treatment of Prostate Cancer. Clin Cancer Res. 2009;15(15):4799–805. https://doi.org/10.1158/1078-0432.CCR-08-0125
47. Shorning BY, Dass MS, Smalley MJ, Pearson HB. The PI3K-AKT-mTOR Pathway and Prostate Cancer: At the Crossroads of AR, MAPK, and WNT Signaling. Int J Mol Sci. 2020;21(12):4507. https://doi.org/10.3390/ijms21124507
48. Culig Z, Hobisch A, Cronauer MV, Radmayr C, Trapman J, Hittmair A, et al. Androgen receptor activation in prostatic tumor cell lines by insulin-like growth factor-I, keratinocyte growth factor, and epidermal growth factor. Cancer Res. 1994;54(20):5474–8.
49. Bookstein R. Tumor suppressor genes in prostate cancer. Prostate Cancer: Springer; 2001. p. 61–94. https://doi.org/10.1385/1-59259-009-8:61
50. McMenamin ME, Soung P, Perera S, Kaplan I, Loda M, Sellers WR. Loss of PTEN expression in paraffin-embedded primary prostate cancer correlates with high Gleason score and advanced stage. Cancer Res. 1999;59(17):4291–6.
51. Thienger P, Rubin MA. Prostate cancer hijacks the microenvironment. Nat Cell Biol. 2021;23(1):3–5. https://doi.org/10.1038/s41556-020-00616-3
52. Gevaert T, Van Eycke Y-R, Vanden Broeck T, Van Poppel H, Salmon I, Rorive S, et al. The potential of tumour microenvironment markers to stratify the risk of recurrence in prostate cancer patients. PLOS One. 2021;15(12):e0244663. https://doi.org/10.1371/journal.pone.0244663
53. Chen S, Zhu G, Yang Y, Wang F, Xiao Y-T, Zhang N, et al. Single-cell analysis reveals transcriptomic remodellings in distinct cell types that contribute to human prostate cancer progression. Nat Cell Biol. 2021;23(1):87–98. https://doi.org/10.1038/s41556-020-00613-6
54. Ganguly SS, Li X, Miranti CK. The Host Microenvironment Influences Prostate Cancer Invasion, Systemic Spread, Bone Colonization, and Osteoblastic Metastasis. Front Oncol 2014;4(364). https://doi.org/10.3389/fonc.2014.00364
55. McKenzie S, Kyprianou N. Apoptosis evasion: the role of survival pathways in prostate cancer progression and therapeutic resistance. J Cell Biochem. 2006;97(1):18–32. https://doi.org/10.1002/jcb.20634
56. Debatin K-M, Krammer PH. Death receptors in chemotherapy and cancer. Oncogene. 2004;23(16):2950–66. https://doi.org/10.1038/sj.onc.1207558
57. Krammer PH. CD95's deadly mission in the immune system. Nature. 2000;407(6805):789–95. https://doi.org/10.1038/35037728
58. DiPaola RS, Patel J, Rafi MM. Targeting apoptosis in prostate cancer. Hematol Oncol Clin North Am 2001;15(3):509–24. https://doi.org/10.1016/S0889-8588(05)70229-X
59. Hengartner MO. The biochemistry of apoptosis. Nature. 2000;407(6805):770–6. https://doi.org/10.1038/35037710
60. McCarty MF. Targeting multiple signaling pathways as a strategy for managing prostate cancer: multifocal signal modulation therapy. Integr Cancer Ther. 2004;3(4):349–80. https://doi.org/10.1177/1534735404270757
61. Navone NM, Troncoso P, Pisters LL, Goodrow TL, Palmer JL, Nichols WW, et al. p53 protein accumulation and gene mutation in the progression of human prostate carcinoma. J Natl Cancer Inst. 1993;85(20):1657–69. https://doi.org/10.1093/jnci/85.20.1657

4

Current Diagnostics for Prostate Cancer

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Doi: https://doi.org/10.36255/exonpublications.prostatecancer.diagnostics.2021

Abstract: How prostate cancer is diagnosed and staged is an ever-evolving field. It plays a fundamental role in ensuring the appropriate therapeutic options are offered to the patient whilst preventing overdiagnosis and overtreatment. Despite the numerous advances in the field, a suspicion of prostate cancer continues to arise from digital rectal examination and measurement of serum prostate specific antigen (PSA). Additional derivatives of serum PSA along with urinary biomarkers and multiparametric magnetic resonance imaging can then help to risk stratify patients in order to appropriately counsel them on the risks and benefits of a prostate biopsy. After a diagnosis of prostate cancer is reached, further staging may be required and can be achieved by a variety of imaging techniques such as computed tomography (CT), bone scintigraphy, and prostate specific membrane antigen-based positron-emission tomography/CT. In this chapter, we review the current role of these and other diagnostic tools in prostate cancer.

Keywords: diagnosis; imaging; prostate biopsy; prostate cancer gene 3; prostate-specific antigen

In: *Prostate Cancer*. Bott SRJ, Ng KL (Editors). Exon Publications, Brisbane, Australia. ISBN: 978-0-6450017-5-4; Doi: https://doi.org/10.36255/exonpublications.prostatecancer.2021

INTRODUCTION

Diagnostic tools for prostate cancer have undergone significant advancements in recent years to improve the accuracy of prostate cancer detection and avoid over-diagnosis and subsequent overtreatment. Despite this, a suspicion of prostate cancer continues to arise from a raised serum prostate specific antigen (PSA) level, and/or a digital rectal examination (DRE). However, an elevated PSA alone should no longer necessitate a prostate biopsy. The use of diagnostic adjuncts can help to predict the presence of clinically significant prostate cancer thereby avoiding unnecessary biopsies in a proportion of patients.

DIGITAL RECTAL EXAMINATION (DRE)

DRE can be used as an inexpensive diagnostic tool to check the prostate for cancer and to give an assessment of the prostate volume. It has the ability to detect prostate cancer with a volume of >0.2ml, if situated in the posterior peripheral zone, and can be used to raise suspicion irrespective of PSA. However, there is a high degree of interobserver variability, and a normal DRE does not eliminate the risk of a significant prostate cancer (1). An historical prospective multicenter trial found 18% of prostate cancers were detected solely by DRE (2), nowadays this figure is thought to be less. Nevertheless, an abnormal DRE is an indication for a prostate biopsy irrespective of the PSA.

PROSTATE-SPECIFIC ANTIGEN (PSA)

PSA is, broadly speaking, an organ-specific glycoprotein secreted by the prostatic epithelium which may be elevated in a variety of conditions, both benign and malignant. Higher levels of PSA indicate a greater likelihood of prostate cancer. A PSA cut-off of ≤4ng/ml was originally proposed as a normal level in men aged 50–70 years. However, analysis of men with a PSA level of ≤4.0ng/ml in the Prostate Cancer Prevention Trial (PCPT) found 15% had clinically significant prostate cancer (3). Therefore, the ability to detect prostate cancer at any PSA level means that no cut-off thresholds for PSA can be used with absolute confidence. Furthermore, a single elevated PSA reading cannot be relied upon due to normal biological fluctuations. A population-based study found that 30% of men with an abnormal PSA had a return to normal PSA on their next reading (4). This highlights the importance of obtaining a confirmatory PSA reading a few weeks after the first reading. The unreliability of PSA means instead the urologist must take into consideration additional factors to determine if the patient should proceed to biopsy, which may include PSA derivatives.

Age-adjusted PSA

Serum PSA readings do not account for the normal age-related PSA changes. The Olmstead county population study demonstrated that serum PSA increases with

TABLE 1 **Recommended age specific serum PSA reference ranges (5)**

Age (years)	Serum PSA reference range ng/ml
40 – 49	0 – 2.5
50 – 59	0 – 3.5
60 – 69	0 – 4.5
70 – 79	0 – 6.5

age and recommended age-specific reference ranges (Table 1) (5). Therefore, if the decision to proceed to further diagnostic tests for prostate cancer is being based solely on a PSA reading, the patients age should be accounted for in order to appropriately counsel them and avoid an unnecessary biopsy.

PSA density

In addition to changes in PSA with age, the Olmstead county population study also demonstrated an increase in PSA with increasing prostate volume (5). To account for this, PSA density can be calculated as the total PSA divided by prostate volume. An increased PSA density is associated with a higher risk of prostate cancer, with a generally agreed cut off value of between 0.12–0.15 ng/ml/cc (6). A prospective multi-center study in patients undergoing an extended template biopsy has found PSA density to be more predictive than total PSA for detecting prostate cancer (7).

PSA kinetics

Changes in PSA over time can be assessed as PSA velocity (change in PSA over time, ng/ml/year) and PSA doubling time (number of months for the PSA to increase two-fold). Whilst PSA kinetics are useful for prognostic purposes after patients have received treatment, they currently have no role in the diagnostic setting (8).

Free and total PSA

Total PSA readings include the sum of all detectable forms of PSA, including PSA bound to protease inhibitors and free PSA. For reasons that are unclear, the percentage of free PSA has been demonstrated to be lower in patients with prostate cancer compared to those with benign disease (9). A multi-center prospective study evaluated men with a benign prostate gland on palpation and a total PSA level of 4 to 10 ng/ml. The study found the probability of prostate cancer in men aged 65 to 75 years was 55% when the free/total (f/t) PSA ratio was 0.1 and reduced to just 9% when the f/t PSA ratio was >0.25 (10). Therefore, in these select patients with a benign prostate gland and PSA of 4 to 10 ng/ml measuring

free PSA may help to avoid unnecessary imaging or biopsy; but it should be used cautiously as it can be affected by other factors including prostate volume and most patients' f/t PSA ratio falls between 0.1 and 0.25 (11).

ADDITIONAL SERUM TESTS

Additional assays are now commercially available measuring a panel of kallikreins. The use of these tests aims to reduce the number of unnecessary prostate biopsies.

Prostate health index

The prostate health index (Phi) test uses a formula to combine the results of total PSA, free PSA and [-2]proPSA ([-2]proPSA/free PSA x √tPSA). It has been shown to have greater specificity and sensitivity than any of its individual components (12). Furthermore, it has been demonstrated to improve the prediction of clinically significant prostate cancer (aggressive histopathology per Epstein criteria or ≥ Gleason 7) in men with a PSA between 4 and 10 ng/ml (13). The use of Phi has the potential to reduce unnecessary biopsies; however, it has not been widely adopted partly due to the pre-analytical stability of [-2]proPSA. For an accurate [-2]proPSA reading, it is recommended that the serum is separated within 3 hours of the sample being taken as the reading increases with clotting time (14).

Four kallikrein score

Similar to the Phi test, the 4 Kallikrein (4K) score has also been shown to be a predictor for prostate cancer which can be used to avoid unnecessary biopsies (15,16). It combines four kallikrein markers (total PSA, free PSA, intact PSA and kallikrein-like peptidase 2 [hK2]) with patient age, DRE findings and prior biopsy status. A direct comparison of the 4K score and Phi found both tests to be equally predictive of prostate cancer and clinically significant prostate cancer (17).

URINE TESTS

In addition to serum tests, several urinary biomarkers for prostate cancer have been described. These include urinary measurements of prostate cancer gene 3, TMPRSS2:ERG, and SelectMDX test.

Prostate cancer gene 3

Prostate cancer gene 3 (PCA3) is a prostate specific non-coding mRNA that is over expressed in prostate cancer and detectable in urine collected after prostatic massage (18). Initial investigations into the use of PCA3 were performed in men with a previous negative biopsy and persistently elevated PSA levels. These early

studies suggested that using a PCA3 cut off score of 35, the test had a sensitivity of 58% and specificity of 72% and was superior to PSA in predicting the biopsy outcome (19–21). However, the ability of the test to predict clinically significant prostate cancers found variable results. Fewer studies have evaluated the use of PCA3 to direct the need for an initial biopsy. One prospective multicenter study in men with a PSA between 2.5 and 10 ng/ml found a sensitivity of 64% and specificity of 76% and similarly found it superior to PSA in predicting biopsy outcome (22). However, further research is still required in the biopsy naïve patient to understand the use of PCA3 in this setting. Consequently, whilst initial research suggests that PCA3 may be useful in predicting the presence of prostate cancer, particularly in patients that have had a previous benign biopsy, it remains unclear whether it can be accurately used to detect clinically significant disease, what cut off levels should be used, and with the extra expense of performing the test, what clinical benefit it truly offers (23).

TRANSMEMBRANE SERINE PROTEASE 2:ERG

The *ERG* gene is a transcription factor of the ETS family which has been observed to be overexpressed in prostate cancer as a result of its fusion to the transmembrane protease serine 2 gene (*TMPRSS2*) (24). *TMPRSS2:ERG* fusion transcripts can be detected in urine with a sensitivity of 37% and specificity of 93% (25). Further studies have shown improved diagnostic ability when combined with the PCA3 test (Michigan-Prostate score [MiPS]) (26). However, this is still under investigation and it is likely that the discovery of *TMPRSS2:ERG* will have a bigger role as a potential therapeutic target than for diagnostics.

SelectMDX test

Similar to PCA3 and *TMPRSS2:ERG,* the SelectMDX test is based on the presence of mRNA biomarkers in urine namely *HOXC6* and *DLX1*. Combining the presence of these biomarkers with traditional clinical risk factors (PSA, PSA density, DRE, age, history of prostate biopsy and family history), the SelectMDX test has the ability to detect clinically significant prostate cancer (27). Further analysis has demonstrated that the use of SelectMDX may lead to a reduction in unnecessary biopsies and overtreatment (28). However, with the advent of prostate magnetic resonance imaging (MRI), a clear role for all these urinary biomarkers in prostate cancer diagnostics is uncertain. Future research will need to focus on how these biomarkers may be effectively integrated to avoid unnecessary and costly imaging.

IMAGING

The role of imaging in prostate cancer diagnostics is rapidly evolving and can be used to identify clinically significant prostate cancers and avoid unnecessary biopsies.

Transrectal ultrasound (TRUS)

Prostate cancer can appear as a hypoechoic lesion on conventional B-mode TRUS; however, this is a non-specific finding. A large prospective study found no significant difference in the detection of prostate cancer from biopsies of patients with or without hypoechoic lesions (25.5% versus 25.4%) (29). This indicates a hypoechoic lesion itself is not associated with an increase in cancer prevalence and B-mode TRUS alone is not diagnostic of prostate cancer. Nevertheless, it serves a vital purpose in identifying the prostate in order to perform biopsies.

Additional variations in ultrasound (US) imaging have also been assessed for their usefulness in diagnosing prostate cancer. Color doppler US (CDUS) measures blood flow and therefore has the potential to detect prostate cancer as a result of increased tumor vasculature. An early evaluation of CDUS found it was able to diagnose up to 70% of prostate cancers but generally performed better in high-grade disease and when used in combination with the conventional B-mode TRUS (30). However, a further study has shown the use of CDUS in targeted prostate biopsies did not improve prostate cancer detection rates when compared with standard TRUS (31). Contrast enhanced US (CEUS) uses microbubble contrast agents to detect increased microvasculature in the prostate. Its use in detecting prostate cancer has been shown to improve the sensitivity when compared to unenhanced CDUS (32). Sonoelastography is based on the principle that there are significant differences in the elastic properties of benign and malignant prostate tissue. The technique estimates the response of tissues under harmonic mechanical excitation using Doppler ultrasound to detect areas of abnormal stiffness (33). The initial study investigating its use found sonoelastography was able to detect 84.1% of prostate cancers (34).

Whilst each of these US techniques has shown promise in initial studies to improve the detection of prostate cancer, combined imaging is reported to offer the most benefit. Multiparametric US (mpUS) consisting of a combination of B-mode, sonoelastography and CEUS improved the sensitivity for clinically significant prostate cancer to 74% from 55%, 55% and 59%, respectively (35). Nevertheless, the use of US in prostate cancer diagnostics is unclear particularly with the recent evolving role of multiparametric-MRI (mp-MRI) which is more accurate than mpUS (36).

Micro-ultrasound is the only US technique that has shown promise in rivalling mp-MRI. Traditional TRUS operates at frequencies of 6–9 MHz whilst micro-ultrasound is a new modality that operates at 29 MHz. This improves image resolution by 300% allowing for the detection of subtle changes in ductal anatomy. Early results of this technique have demonstrated an improvement in the detection of clinically significant prostate cancer and that it may be able to detect lesions missed on multiparametric-MRI (mp-MRI) (37,38). Although further research is required to understand the exact role micro-ultrasound will have in prostate cancer diagnostics.

Multiparametric magnetic resonance imaging

The European Society of Urogenital Radiology recommends mp-MRI for the detection of prostate cancer should include a combination of high-resolution T2 weighted images and at least two functional MRI techniques; diffusion weighted

imaging (DWI) and dynamic contrast enhanced (DCE) imaging (39). Prostate cancer typically manifests as a round low signal intensity focus on T2-weighted MRI, high signal intensity on DWI at high b-values and classically demonstrates early enhancement on DCE-MRI. The Prostate Imaging-Reporting and Data System (PI-RADS) provides a structured way to report each lesion by allocating a score between 1 and 5 that predicts its chance of being a clinically significant prostate cancer; with 5 indicating a very high likelihood for the presence of clinically significant prostate cancer (40). A meta-analysis assessing the diagnostic accuracy of mp-MRI for prostate cancer found it to have high specificity and sensitivity, 88% and 74%, with a variable but high negative predictive value ranging from 65–94% (41). Furthermore, a comparison of pre-operative MRI to radical prostatectomy histopathology found prostate cancer detection rates increased with both tumor volume and increasing Gleason score (42). One of the main uses of mp-MRI is to identify a target to biopsy to improve the detection of clinically significant prostate cancers (43). This will be discussed further in the chapter along with its use in staging. In addition, a prebiopsy mp-MRI can also be used to avoid undertaking biopsies in patients with no visible lesions. The PROMIS trial found that using a mp-MRI and only performing a prostate biopsy on patients with PI-RADS lesions of ≥3 could have avoided a biopsy in 27% of patients (44).

RISK CALCULATORS

The use of risk calculators can help to combine diagnostic tests to predict an individual patients' risk of clinically significant prostate cancer and potentially reduce unnecessary investigations. One such validated risk calculator is that developed from the PCPT cohort. The PCPT predictive model was initially developed to combine the patients' age, race, family history, serum PSA, DRE and prior biopsy status to produce a risk score for having both low- and high-grade prostate cancer on a biopsy (45). Further developments now provide the option to include free PSA, urinary PCA3 and *TMPRSS2:ERC* into the PCPT calculator (46,47). Other risk calculators also include mp-MRI findings. A systematic review has identified that over 100 prediction models exist in the literature, although not all of these have been validated and currently no single model has shown superiority over another (48).

PROSTATE BIOPSY

The modern era of prostate biopsies began with the systematic sextant method in which initially 6 and subsequently 12 ultrasound guided biopsies were taken from 6 sites (apex, middle and base of each lobe) (49). Currently, TRUS guided prostate biopsy can be performed via either a transrectal or transperineal approach. A meta-analysis comparing the two biopsy approaches found the diagnostic accuracy to be comparable, however, the transperineal approach was associated with a lower risk of fever and rectal bleeding (50). Following the publication of the landmark PROMIS study, a prebiopsy mp-MRI is now the gold standard to perform

targeted biopsies (44). A subsequent Cochrane review found this approach increases the number of significant cancers detected while reducing the number of insignificant cancers diagnosed (43). Different methods for performing targeted biopsies of lesions identified on mp-MRI exist; direct in-bore targeted biopsy, fusion biopsy, and cognitive targeted biopsy.

Direct in-bore targeted biopsy

Direct in-bore MRI targeted biopsy in which the biopsies are performed in the MR scanner using real time MRI guidance. A prospective matched cohort study comparing this technique with a 10-core TRUS biopsy found a significantly improved correlation with histology at radical prostatectomy (88% versus 55%) (51). However, this is a labor intense and costly procedure, taking up 2–3 hours of scanning time. It requires administration of a general anesthetic with the patient in the scanner potentially creating difficulty with airway management.

MRI fusion biopsy

An MRI-transperineal or transrectal fusion target biopsy is where software is used to merge the MRI image of the prostate with the TRUS image in real time to accurately direct biopsies. Several different systems are available including Artemis, Biopsee and Koelis Trinity. The system records the site of biopsy confirming that the selected target has been sampled and is useful for future reference. This approach takes some extra time as the prostate and lesion requires contouring but is faster and less expensive than the direct in-bore biopsy technique. The main potential source of error is in the co-registration of the MRI and TRUS images. The prostate images are obtained in different positions; MRI in supine and TRUS either in the left lateral or lithotomy with the hips flexed which rotates the prostate within the pelvis. Image registration is either rigid or elastic. Rigid image registration overlays the MRI images onto the TRUS images without any adjustment for possible deformation during the procedure such as from patient movement. Whilst elastic registration does compensate for this deformation and, therefore, would be anticipated to be more accurate. However, a meta-analysis comparing rigid and elastic registration found no significant difference in the detection of clinically significant prostate cancer (52).

Cognitive targeted biopsy

Finally, cognitive targeted biopsy or visual registration are where the MRI images are reviewed by the urologist who then performs the biopsies, either via a transperineal or transrectal route, using TRUS guidance aiming to sample the general location of the suspicious lesion. This is the simplest, fastest, and cheapest method to perform MRI-targeted biopsies. However, the accuracy is highly dependent on operator experience and training requiring good knowledge of prostate zonal anatomy on both MRI and TRUS images. Furthermore, in cases of negative template biopsy for quality control there is no ability to check whether the target was sampled (53). Despite this, a comparison of cognitive targeted to systematic biopsies found no statistically significant difference in the detection

of clinically significant prostate cancer and found fewer insignificant cancers were detected (54).

What is the preferred biopsy approach?

There is clear evidence that MRI targeted biopsies improve the detection of clinically significant prostate cancer and results in fewer insignificant lesions being detected. So far studies have failed to demonstrate any of the different MRI targeting techniques described to be superior to another (55,56). Targeted biopsies can be taken via a transperineal or transrectal approach with the former having a reduced risk of sepsis (50). Other factors to consider when performing a biopsy include anesthetic and position. Biopsies can be performed under general or local anesthetic. The local anesthetic technique has been shown to have good patient tolerability without the associated risks of a general anesthetic and with reduced operative time and patient recovery (57). Furthermore, biopsies under local anesthetic can be performed in the lithotomy or left lateral decubitus position, with the latter associated with improved pain scores (58).

STAGING

Once a diagnosis of prostate cancer has been reached, the patient requires clinical staging in order to direct the appropriate treatment.

Multiparametric magnetic resonance imaging

In addition to directing the need for a prostate biopsy, mp-MRI can be used for local staging of prostate cancer. T2-weighted imaging can be used to look for extracapsular extension (ECE) (T3a), seminal vesicle invasion (SVI) (T3b) and invasion into other organs (T4). Pooled data from a meta-analysis has demonstrated mp-MRI has high specificity but poor sensitivity in detecting ECE, 91% and 57%, and SVI, 96% and 58%, respectively (59). The use of mp-MRI to assess the prostate for suspicious lesions also indirectly provides an assessment of nodal disease. However, similar to its use in local staging, mp-MRI has also been shown to have poor sensitivity for the detection of nodal disease. A meta-analysis found a pooled sensitivity of 39% and specificity of 82% with significant study heterogeneity (60). Accordingly, mp-MRI can therefore not be completely relied upon for local staging for the presence of lymph node metastases.

Computed tomography

The use of computed tomography (CT) in the detection of lymph node metastases has also been shown to be an unreliable method. Similar to mp-MRI, a meta-analysis found a good specificity at 82% but a poor sensitivity of 42% (60). The main drawback in the use of CT and mp-MRI to detect lymph node metastases is their reliance on nodal enlargement which is not always present (61).

Choline positron emission tomography CT

The use of choline positron emission tomography (PET) CT is based on high uptake of the radiotracer believed to be due to the increase in membrane phosphatidylcholine in cancer cells (62). Its use in prostate cancer diagnostics has largely been evaluated in its ability to detect lymph node metastases which has found variable results. However, its utilization in high-risk prostate cancer has demonstrated a significantly improved specificity and sensitivity suggesting it may be useful under these conditions for the detection of nodal metastases (63). Although, with the developments in ^{68}Gallium (^{68}Ga) labelled prostate specific membrane antigen (PSMA) PET-CT, it is unclear whether choline PET-CT will have a role in the future of prostate cancer diagnostics.

Bone scan

Bone metastases are most frequently looked for using a technetium Tc 99m methylene disphophonate (Tc 99m MDP) bone scan. PSA, Gleason score, and clinical stage are all significant predictors of bone metastases. It is suggested that a staging baseline bone scan should be performed in patients with intermediate (PSA 10–20 ng/ml or Gleason score 7 or cT2b) or high-risk prostate cancer (PSA >20ng/ml or Gleason score 8–10 or cT2c/3/4). By using these criteria, it was found that staging baseline bone scan could be avoided in approximately 81% of patients with a negative predictive value of 99.6% (64).

Prostate specific membrane antigen-based PET CT

^{68}Ga PSMA PET-CT shows great promise in improving prostate cancer diagnostics. PSMA is over-expressed on the cell membrane of nearly all prostate cancer cells with expression levels increasing according to the stage and grade of tumor (65). A meta-analysis comparing ^{68}Ga PSMA PET CT with MRI for the diagnosis of lymph node metastases in patients with intermediate or high-risk prostate cancer found ^{68}Ga PSMA PET CT to have a higher sensitivity (65% versus 41%) (66). A further meta-analysis has also demonstrated ^{68}Ga PSMA PET-CT to have the highest sensitivity and specificity for the diagnosis of bone metastases when compared with choline PET-CT, MRI, and bone scintigraphy (67). A recent multicenter randomized study also found ^{68}Ga PSMA PET-CT in men with high-risk prostate cancer (Gleason grade group 3–5, PSA ≥20 or clinical stage ≥T3) was superior to bone scan and CT, with a 92% accuracy. Importantly, this improved method of staging resulted in more frequent changes to the patients' management plan, and it therefore has the potential to offer the most appropriate first line therapy in addition to avoiding unnecessary treatment (68).

CONCLUSION

The integration of these diagnostic tools for prostate cancer enables the urologist to risk stratify patients and appropriately direct the diagnostic path. There have been significant improvements in the detection of clinically significant prostate

cancer in addition to preventing overdiagnosis as well as improvements in staging. However, further advances to improve the sensitivity of staging investigations and streamlining of the pathway are required to make this both clinically and cost-effective.

Conflict of interest: The authors declare no potential conflict of interest with respect to research, authorship and/or publication of this chapter.

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REFERENCES

1. Gosselaar C, Kranse R, Roobol MJ, Roemeling S, Schröder FH. The interobserver variability of digital rectal examination in a large randomized trial for the screening of prostate cancer. Prostate. 2008; 68(9): 985–93. https://doi.org/10.1002/pros.20759
2. Richie JP, Catalona WJ, Ahmann FR, Hudson MA, Scardino PT, Flanigan RC et al. Effect of patient age on early detection of prostate cancer with serum prostate-specific antigen and digital rectal examination. Urology. 1993; 42(4): 365–74. https://doi.org/10.1016/0090-4295(93)90359-I
3. Thompson IM, Pauller DK, Goodman PJ, Tangen CM, Scott Lucia M, Parnes HL et al. Prevalence of prostate cancer among men with a prostate-specific antigen level < or = 4.0ng per millilitre. N Engl J Med. 2004; 350(22): 2239–46. https://doi.org/10.1056/NEJMoa031918
4. Eastham JA, Riedel E, Scardino PT, Shike M, Fleisher M, Schatzkin A et al. Variation of serum prostate specific antigen levels. JAMA. 2003; 289(20): 2695–700. https://doi.org/10.1001/jama.289.20.2695
5. Oesterling JE, Jacobsen SJ, Chute CG, Guess HA, Girman CJ, Panser LA et al. Serum prostate-specific antigen in a community-based population of healthy men. Establishment of age-specific reference ranges. JAMA. 1993; 270(7): 860–4. https://doi.org/10.1001/jama.1993.03510070082041
6. Benson MC, Olsson CA. Prostate specific antigen and prostate specific antigen density. Roles in patient evaluation and management. Cancer. 1994; 74(6): 1667–73. https://doi.org/10.1002/1097-0142(19940915)74:6<1667::AID-CNCR2820740605>3.0.CO;2-2
7. Jue JS, Barboza MP, Prakash NS, Venkatramani V, Sinha VR, Pavan N et al. Re-examining prostate-specific antigen (PSA) density: defining the optimal PSA range and patients for using PSA density to predict prostate cancer using extended template biopsy. Oncology. 2017; 105: 123–8. https://doi.org/10.1016/j.urology.2017.04.015
8. Vickers AJ, Brewster SF. PSA Velocity and Doubling Time in Diagnosis and Prognosis of Prostate Cancer. Br J Med Surg Urol. 2012; 5(4): 162–8. https://doi.org/10.1016/j.bjmsu.2011.08.006
9. Catalona WJ, Smith DS, Wolfert RL, Wang TJ, Rittenhouse HG, Ratliff TL, et al. Evaluation of percentage of free serum prostate-specific antigen to improve specificity of prostate cancer screening. JAMA. 1995; 274(15): 1214–20. https://doi.org/10.1001/jama.1995.03530150038031
10. Catalona WJ, Partin AW, Slawin KM, Brawer MK, Flanigan RC, Patel A et al. Use of the percentage of free prostate-specific antigen to enhance differentiation of prostate cancer from benign prostatic disease. JAMA. 1998; 279(19): 1542–7. https://doi.org/10.1001/jama.279.19.1542
11. Stephan C, Lein M, Jung K, Schnorr D, Loening SA. The influence of prostate volume on the ratio of free to total prostate specific antigen in serum of patients with prostate carcinoma and benign prostate hyperplasia. Cancer. 1997; 79(1): 104–9. https://doi.org/10.1002/(SICI)1097–0142(19970101)79:1<104::AID-CNCR15>3.0.CO;2–8
12. Catalona WJ, Partin AW, Sanda MG, Wei JT, Klee GG, Bangma CH et al. A multicentre study of [-2] pro-prostate specific antigen combined with prostate specific antigen and free prostate specific

antigen for prostate cancer detection in the 2.0 to 10.0ng/ml prostate specific antigen range. J Urol. 2011; 185(5): 1650–5. https://doi.org/10.1016/j.juro.2010.12.032
13. Loeb S, Sanda MG, Broyles DL, Shin SS, Bangma CH, Wei JT et al. The prostate health index selectively identifies clinically significant prostate cancer. J Urol. 2015; 193(4): 1163–9. https://doi.org/10.1016/j.juro.2014.10.121
14. Semjonow A, Köpke T, Eltze E, Pepping-Schefers B, Bürgel H, Darte C. Pre-analytical in-vitro stability of [-2]proPSA in blood and serum. Clin Biochem. 2010; 43(10–11): 926–8. https://doi.org/10.1016/j.clinbiochem.2010.04.062
15. Vickers AJ, Cronin AM, Aus G, Pihl C, Becker C, Pettersson K et al. A panel of kallikrein markers can reduce unnecessary biopsy for prostate cancer: data from the European Randomized study of prostate cancer screening in Göteborg, Sweden. BMC Med. 2008; 6: 19. https://doi.org/10.1186/1741-7015-6-19
16. Parekh DJ, Punnen S, Sjoberg DD, Asroff SW, Bailen JL, Cochran JS et al. A multi-institutional prospective trial in the USA confirms that the 4Kscore accurately identifies men with high-grade prostate cancer. Eur Urol. 2015; 68(3): 464–70. https://doi.org/10.1016/j.eururo.2014.10.021
17. Nordström T, Vickers A, Assel M, Lilja H, Grönberg H, Eklund M. Comparison between the four-kallikrein panel and prostate health index for predicting prostate cancer. Eur Urol. 2015; 68(1): 139–46. https://doi.org/10.1016/j.eururo.2014.08.010
18. Hessels D, Gunnewiek JMTK, Oort IV, Karthaus HFM, van Leenders GJL, Balken BV, et al. DD3PCA3-based molecular urine analysis for the diagnosis of prostate cancer. Eur Urol. 2003 Jul; 44(1): 8–16. https://doi.org/10.1016/S0302-2838(03)00201-X
19. Marks LS, Fradet Y, Deras IL, Blase A, Mathis J, Aubin SMJ, et al. PCA3 molecular urine assay for prostate cancer in men undergoing repeat biopsy. Urology. 2007; 69(3): 532–5. https://doi.org/10.1016/j.urology.2006.12.014
20. Deras IL, Aubin SMJ, Blase A, Day JR, Koo S, Partin AW et al. PCA3: A molecular urine assay for predicting prostate biopsy outcome. J Urol. 2008; 179(4): 1587–92. https://doi.org/10.1016/j.juro.2007.11.038
21. Haese A, de la Taille A, van Poppel H, Marberger M, Stenzl A, Mulders PFA et al. Clinical utility of the PCA3 urine assay in European men scheduled for repeat biopsy. Eur Urol. 2008; 54(5): 1081–8. https://doi.org/10.1016/j.eururo.2008.06.071
22. De la Taille A, Irani J, Graefen M, Chun F, de Reijke T, Kil P et al. Clinical evaluation of the PCA3 assay in guiding initial biopsy decisions. J Urol. 2011; 185(6): 2119–25. https://doi.org/10.1016/j.juro.2011.01.075
23. Nicholson A, Mahon J, Boland A, Beale S, Dwan K, Fleeman N et al. The clinical effectiveness and cost-effectiveness of the PROGENSA® prostate cancer antigen 3 assay and the Prostate Health Index in the diagnosis of prostate cancer: a systematic review and economic evaluation. Health Technol Assess. 2015; 19(87): i–xxxi, 1–191. https://doi.org/10.3310/hta19870
24. Tomlins SA, Mehra R, Rhodes DR, Smith LR, Roulston D, Helgeson BE et al. TMPRSS2:ETV4 gene fusions define a third molecular subtype of prostate cancer. Cancer Res. 2006; 66(7): 3396–400. https://doi.org/10.1158/0008-5472.CAN-06-0168
25. Hessels D, Smit FP, Verhaegh GW, Witjes JA, Corel EB, Schalken JA. Detection of TMPRSS2-ERG fusion transcripts and prostate cancer antigen 3 in urinary sediments may improve diagnosis of prostate cancer. Clin Cancer Res. 2007; 13(17): 5103–8. https://doi.org/10.1158/1078-0432.CCR-07-0700
26. Tomlins SA, Day JR, Lonigro RJ, Hovelson DH, Siddiqui J, Kunju LP et al. Urine TMPRSS2:ERG plus PCA3 for individualized prostate cancer risk assessment. Eur Urol. 2016; 70(1): 45–53. https://doi.org/10.1016/j.eururo.2015.04.039
27. Van Neste L, Hendriks RJ, Dijkstra S, Trooskens G, Cornel EB, Jannink SA, et al. Detection of high-grade prostate cancer using a urinary molecular biomarker-based risk score. Eur Urol. 2016r; 70(5): 740–8. https://doi.org/10.1016/j.eururo.2016.04.012
28. Dijkstra S, Govers TM, Hendriks RJ, Schalken JA, Criekinge WV, Van Neste L, et al. Cost-effectiveness of a new urinary biomarker-based risk score compared to standard of care in prostate cancer diagnostics - a decision analytical model. BJU Int. 2017; 120(5): 659–65. https://doi.org/10.1111/bju.13861

29. Onur R, Littrup PJ, Pontes JE, Bianco FJ. Contemporary impact of transrectal ultrasound lesions for prostate cancer detection. J Urol. 2004; 172(2): 512–4. https://doi.org/10.1097/01.ju.0000131621.61732.6b
30. Shigeno K, Igawa M, Shiina H, Wada H, Yoneda T. The role of colour Doppler ultrasonography in detecting prostate cancer. BJU Int. 2000; 86: 229–33. https://doi.org/10.1046/j.1464-410x.2000.00829.x
31. Taverna G, Morandi G, Seveso M, Giusti G, Benetti A, Colombo P, et al. Colour Doppler and microbubble contrast agent ultrasonography do not improve cancer detection rate in transrectal systematic prostate biopsy sampling. BJU Int. 2011; 108(11): 1723–7. https://doi.org/10.1111/j.1464-410X.2011.10199.x
32. Roy C, Buy X, Lang H, Saussine C, Jacqmin D. Contrast enhanced color doppler endorectal sonography of prostate: efficiency for detecting peripheral zone tumors and role for biopsy procedure. J Urol. 2003; 170(1): 69–72. https://doi.org/10.1097/01.ju.0000072342.01573.8d
33. Hoyt K, Castaneda B, Zhang M, Nigwekar P, di Sant'Agnese PA, Joseph JV et al. Tissue elasticity properties as biomarkers for prostate cancer. Cancer Biomark. 2008; 4(4–5): 213–25. https://doi.org/10.3233/CBM-2008-44-505
34. Kõnig K, Scheipers U, Pesavento A, Lorenz A, Ermert H, Senge T. Initial experiences with real-time elastography guided biopsies of the prostate. 2005; 174(1): 115–7. https://doi.org/10.1097/01.ju.0000162043.72294.4a
35. Mannaerts CK, Wildeboer RR, Remmers S, van Kollenburg RAA, Kajtazovic A, Hagemann J, et al. Multiparametric ultrasound for prostate cancer detection and localization: correlation of B-mode, shear wave elastography and contrast enhanced ultrasound with radical prostatectomy specimens. J Urol. 2019; 202(6): 1166–73. https://doi.org/10.1097/JU.0000000000000415
36. Drudi FM, Cantisani V, Angelini F, Ciccariello M, Messineo D, Ettorre E, et al. Multiparametric MRI versus Multiparametric US in the detection of prostate cancer. Anticancer Res. 2019; 39(6): 3101–10. https://doi.org/10.21873/anticanres.13446
37. Rohrbach D, Wodlinger B, Wen J, Mamou J, Feleppa E. High-frequency quantitative ultrasound for imaging prostate cancer using a novel micro-ulltrasound scanner. Ultasound Med Biol. 2018; 44(7): 1341–54. https://doi.org/10.1016/j.ultrasmedbio.2018.02.014
38. Abouassaly R, Klein EA, El-Shefai A, Stephenson A. Impact of using 29 MHz high-resolution micro-ultrasound in real-time targeting of transrectal prostate biopsies: initial experience. World J Urol. 2020; 38(5): 1201–6. https://doi.org/10.1007/s00345-019-02863-y
39. Barentsz JO, Richenberg J, Clements R, Choyke P, Verma S, Villeirs G, et al. ESUR prostate MR guidelines 2012. Eur Radiol. 2012; 22(4): 746–57 https://doi.org/10.1007/s00330-011-2377-y
40. American College of Radiology. Prostate Imaging - Reporting and Data System. 2019. Version 2.1. Available at https://www.acr.org/-/media/ACR/Files/RADS/PI-RADS/PIRADS-V2-1.pdf
41. De Rooij M, Hamoen EHJ, Fütterer JJ, Barentsz JO, Rovers MM. Accuracy of multiparametric MRI for prostate cancer detection: a meta-analysis. AJR Am J Roentgenol. 2014; 202(2): 343–51. https://doi.org/10.2214/AJR.13.11046
42. Bratan F, Niaf E, Melodelima C, Chesnais AL, Souchon R, Mège-Lechevallier F, et al. Influence of imaging and histological factors on prostate cancer detection and localisation on multiparametric MRI: a prospective study. Eur Radiol. 2013; 23(7): 2019–29. https://doi.org/10.1007/s00330-013-2795-0
43. Drost FJH, Osses DF, Nieboer D, Steyerberg EW, Bangma CH, Roobol MJ, et al. Prostate MRI, with or without MRI-targeted biopsy, and systematic biopsy for detecting prostate cancer. Cochrane Database Syst Rev. 2019; 4(4): CD012663. https://doi.org/10.1002/14651858.CD012663.pub2
44. Ahmed HU, Bosaily AE, Brown LC, Gabe R, Kaplan R, Parmar MK, et al. Diagnostic accuracy of multiparametric MRI and TRUS biopsy in prostate cancer (PROMIS): a paired validating confirmatory study. Lancet. 2017; 389(10071): 815–22. https://doi.org/10.1016/S0140-6736(16)32401-1
45. Thompson IM, Ankerst DP, Chi C, Goodman PJ, Tangen CM, Lucia MS et al. Assessing prostate cancer risk: results from the Prostate Cancer Prevention Trial. J Natl Cancer Inst. 2006; 98(8): 529–34. https://doi.org/10.1093/jnci/djj131
46. Ankerst DP, Hoefler J, Bock S, Goodman PJ, Vickers A, Hernandez J et al. Prostate Cancer Prevention Trial risk calculator 2.0 for the prediction of low- vs high-grade prostate cancer. Urology. 2014; 83(6): 1362–7. https://doi.org/10.1016/j.urology.2014.02.035

47. Ankerst DP, Goros M, Tomlins SA, Patil D, Fenng Z, Wei JT et al. Incorporation of urinary prostate cancer antigen 3 and TMPRSS2:ERG into Prostate Cancer Prevention Trial Risk Calculator. Eur Urol Foc. 2019; 5(1): 54–61. https://doi.org/10.1016/j.euf.2018.01.010
48. Louie KS, Seigneurin A, Cathcart P, Sasieni P. Do prostate cancer risk models improve the predictive accuracy of PSA screening? A meta-analysis. Ann Oncol. 2015; 26(5): 848–64. https://doi.org/10.1093/annonc/mdu525
49. Hodge KK, McNeal JE, Terris MK, Stamey TA. Random systematic versus directed ultrasound guided transrectal core biopsies of the prostate. J Urol. 1989; 142(1): 71–4. https://doi.org/10.1016/S0022-5347(17)38664-0
50. Xiang J, Yan H, Li J, Wang X, Chen H, Zheng X. Transperineal versus transrectal prostate biopsy in the diagnosis of prostate cancer: a systematic review and meta-analysis. World J Surg Oncol. 2019; 17(1): 31. https://doi.org/10.1186/s12957-019-1573-0
51. Hambrock T, Hoeks C, Hulsbergen-van de Kaa C, Scheenen T, Fütterer J, Bouwense S, et al. Prospective assessment of prostate cancer aggressiveness using 3-T diffusion-weighted magnetic resonance imaging-guided biopsies versus a systematic 10-core transrectal ultrasound prostate biopsy cohort. Eur Urol. 2012; 61(1): 177–84. https://doi.org/10.1016/j.eururo.2011.08.042
52. Venderink W, de Rooiij M, Sedelaar JPM, Juisman JH, Fütterer HJ. Elastic versus rigid image registration in magnetic resonance imaging-transrectal ultrasound fusion prostate biopsy: a systematic review and meta-analysis. Eur Urol. 2018; 4(2): 219–27. https://doi.org/10.1016/j.euf.2016.07.003
53. Puech P, Ouzzane A, Gaillard V, Betouni N, Renard B, Villers A, et al. Multiparametric MRI-targeted TRUS prostate biopsies using visual registration. Biomed Res Int. 2014; 819360 https://doi.org/10.1155/2014/819360
54. Kasivisvanathan V, Dufour R, Moore CM, Ahmed HU, Abd-Alazeez M, Charman SC, et al. Transperineal magnetic resonance image targeted prostate biopsy versus transperineal template prostate biopsy in the detection of clinically significant prostate cancer. J Urol. 2013; 189(3): 860–6. https://doi.org/10.1016/j.juro.2012.10.009
55. Wegelin O, van Melick HHE, Hooft L, Ruud Bosch JLH, Reitsma HB, Barentsz JO, et al. Comparing three different techniques for magnetic resonance imaging-targeted prostate biopsies: A systematic review of in-bore versus magnetic resonance imaging-transrectal ultrasound fusion versus cognitive registration. Is there a preferred technique? Eur Urol. 2017; 71(4): 517–31. https://doi.org/10.1016/j.eururo.2016.07.041
56. Wegelin O, Exterkate L, van der Leest M, Kummer JA, Vreuls W, de Bruin PC et al. The FUTURE Trial: a multicentre randomised controlled trial on target biopsy techniques based on magnetic resonance imaging in the diagnosis of prostate cancer in patients with prior negative biopsies. Eur Urol. 2019; 75(4): 582–90. https://doi.org/10.1016/j.eururo.2018.11.040
57. Stefanova V, Buckley, Flax S, Spevack L, Hajek D, Tunis A, et al. Transperineeal prostate biopsies using local anesthesia: experience with 1,287 patients. Prostate cancer detection rate, complications and patient tolerability. J Urol. 2019; 201(6): 1121–6. https://doi.org/10.1097/JU.0000000000000156
58. Lodeta B, Lodeta M. Prostate biopsy in the left lateral decubitus position is less painful than prostate biopsy in the lithotomy postion: a randomized controlled trial. Korean J Urol. 2012; 53(2): 87–91. https://doi.org/10.4111/kju.2012.53.2.87
59. De Rooij M, Hamoen EHJ, Witjes JA, Barentsz JO, Rovers MM. Accuracy of magnetic resonance imaging for local staging of prostate cancer: a diagnostic meta-analysis. Eur Urol. 2016; 70(2): 233–45. https://doi.org/10.1016/j.eururo.2015.07.029
60. Hövels AM, Heesakkers RAM, Adang EM, Jager GJ, Strum S, Hoogeveen YL, et al. The diagnostic accuracy of CT and MRI in the staging of pelvic lymph nodes in patients with prostate cancer: a meta-analysis. Clin Radiol. 2008; 63(4): 387–95. https://doi.org/10.1016/j.crad.2007.05.022
61. Tiguert R, Gheiler EL, Tefilli MV, Oskanian P, Banerjee M, Grignon DJ, et al. Lymph node size does not correlate with the presence of prostate cancer metastasis. Urology. 1999; 53(2): 367–71. https://doi.org/10.1016/S0090-4295(98)00518-4
62. Schwarenböck S, Souvatzoglou M, Krause BJ. Choline PET and PET/CT in primary diagnosis and staging of prostate cancer. Theranostics. 2012; 2(3): 318–30. https://doi.org/10.7150/thno.4008

63. Schiavina R, Bianchi L, Bianchii FM, Borghesi M, Pultrone CV, Dababneh H et al. Preoperative staging with 11C-Choline PET/CT is adequately accurate inn patients with very high-risk prostate cancer. Clin Genitourin Cancer. 2018; 16(4): 305–12. https://doi.org/10.1016/j.clgc.2018.05.010
64. Briganti A, Passoni N, Ferrari M, Capitanio U, Suardi N, Gallina A, et al. When to perform bone scan in patients with newly diagnosed prostate cancer: external validation of the currently available guidelines and proposal of a novel risk stratification tool. Eur Urol. 2010 ; 57(4): 551–8. https://doi.org/10.1016/j.eururo.2009.12.023
65. Maurer T, Elber M, Schwaiger M, Gschwend JE. Current use of PSMA-PET in prostate cancer management. Nat Rev Urol. 2016; 13(4): 226–35. https://doi.org/10.1038/nrurol.2016.26
66. Wu H, Xu T, Wang X, Yu YB, Fan ZY, Li DX et al. Diagnostic Performance of 68Gallium labelled prostate-specific membrane antigen positron emission tomography/computed tomography and magnetic resonance imaging for staging the prostate cancer with intermediate or high risk prior to radical prostatectomy: a systematic review and meta-analysis. World J Mens Health. 2020; 38(2): 208–19. https://doi.org/10.5534/wjmh.180124
67. Zhou J, Gou Z, Wu R, Yuan Y, Yu G, Zhao Y. Comparison of PSMA-PET/CT, choline PET/CT, NaF-PET/CT, MRI, and bone scintigraphy in the diagnosis of bone metastases in patients with prostate cancer: a systematic review and meta-analysis. Skeletal Radiol. 2019 ; 48(12): 1915–24. https://doi.org/10.1007/s00256-019-03230-z
68. Hofman MS, Lawrentschuk N, Francis RJ, Tang C,Vela I, Thomas P et al. Prostate-specific membrane antigen PET-CT in patients with high-risk prostate cancer before curative-intent surgery or radiotherapy (proPSMA): a prospective, randomised, multicentre study. Lancet. 2020; 395(10231): 1208–16. https://doi.org/10.1016/S0140-6736(20)30314-7

5

Combination Treatment Options for Castration-Resistant Prostate Cancer

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Doi: https://doi.org/10.36255/exonpublications.prostatecancer.combinationtreatment.2021

Abstract: Prostate cancer is the most commonly diagnosed solid tumor and the second leading cause of cancer-related deaths in men in the United States. While localized prostate cancer has an excellent prognosis for patients, about one-third of patients are diagnosed with high-risk disease, including metastatic cancer. The 5-year survival rate of metastatic prostate cancer is only about 30%. Due to the androgen dependence of prostate cancer cells, androgen-deprivation therapy is the standard of care for metastatic prostate cancer, which includes both surgical and medical approaches. Nevertheless, androgen-deprivation therapy in general

In: *Prostate Cancer*. Bott SRJ, Ng KL (Editors). Exon Publications, Brisbane, Australia. ISBN: 978-0-6450017-5-4; Doi: https://doi.org/10.36255/exonpublications.prostatecancer.2021

is not curative; patients can develop castration-resistant prostate cancer. Despite current chemotherapies, including the utilization of novel androgen signaling inhibitors and immunotherapy, patients still succumb to the disease. Hence, castration-resistant prostate cancer is a lethal disease. Combination treatment is a strategy for treating this lethal disease and thus will be the focus of discussion in this chapter.

Keywords: androgen deprivation therapy; castration-resistant prostate cancer; chemotherapy; combination treatment; immunotherapy

INTRODUCTION

Prostate cancer is the most commonly diagnosed solid tumor, and the second leading cause of cancer-related deaths in men in the United States (1). Patients with localized prostate cancer have an excellent prognosis (2), however, up to 15% of prostate cancer patients are diagnosed with high-risk disease, i.e., disease with prostate-specific antigen (PSA) levels of over 20 ng/mL and Gleason scores of 8 or higher (3). The 5-year survival of patients with metastatic prostate cancer is about 30%.

Androgens are essential for normal prostate development and differentiation but are also involved in prostate cancer initiation and progression. Hence, androgen-deprivation therapy (ADT), which blocks androgen receptor (AR) signaling, is the standard of care for treating metastatic prostate cancer (4, 5). Nevertheless, ADT is not curative; most prostate cancer cells eventually become resistant to ADT, becoming a castration-resistant (CR) phenotype. The CR phenotype of prostate cancer cells can be achieved through a variety of mechanisms, including AR elevation to sustain AR signaling with residual levels of circulating androgens, AR mutation in the ligand-binding site causing receptor promiscuity, truncation of AR structure generating constitutively activated variants, intra-tumoral androgen biosynthesis for intracrine AR activation, and/or ligand-independent AR activation by growth factor signaling pathways, such as ErbB-2 (6–14).

Neuroendocrine (NE)-like prostate cancer cells can also support the CR phenotype. In a normal prostate, authentic NE cells are a minor population; in cancer, it makes up less than 5% of total prostate cancer cases. Nevertheless, up to 60% of CR tumors are found to contain the NE-like cells (15–17), i.e., cells that express NE biomarkers. NE-like cells undergo trans-differentiation from adenocarcinoma cells during therapies, especially prolonged ADT, and can support prostate cancer cell survival and progression under ADT through secretion of growth factors (15–17). Currently, there are no FDA-approved agents that can effectively treat patients with CR prostate cancer, authentic NE prostate cancer, or NE-like prostate cancer. The current FDA-approved drugs either alone or in combination can only extend the life of a patient by a few months. In this chapter, we discuss current treatments and summarize the recent completed combination trials as well as ongoing emerging combination trials for the management of CR prostate cancer.

CURRENT TREATMENT STRATEGIES FOR PROSTATE CANCER

Currently, treating prostate cancer patients is a well-organized roadmap according to FDA guidelines, however, none of these options are curative and the disease will often progress after a short period of time. Below, we discuss the current therapeutic strategies utilized for each stage of prostate cancer including surgery and radiotherapy, ADT, taxanes, and sipuleucel-T.

Surgery and radiotherapy

While watchful waiting and active surveillance are the preferred method of choice for men with certain low-risk prostate cancer (18), several other strategies are available for localized disease. Surgery, as well as external beam radiotherapy (EBRT) and brachytherapy are all common treatment regimens for localized prostate cancer. EBRT is often utilized in patients with high-risk disease, while brachytherapy is effective for patients with low-risk disease (19, 20). Primary surgery is a viable treatment option for prostate cancer and allows for histopathological analysis of the tumor (21). Surgery has been shown to be more beneficial than watchful waiting in terms of mortality, disease progression and metastasis (22). To date, surgery and radiotherapy remain the first line of defense against localized prostate cancer (Figure 1). However, it is important to note that there is no difference in mortality rate between active surveillance, surgery, or radiotherapy for low-risk patients (23). Nevertheless, these treatment options are not 100% effective, as relapse of metastatic disease and progression to CR prostate cancer can occur. Some completed clinical trials are shown in Table 1, while those ongoing emerging combination trials are in Table 2.

Androgen deprivation therapy (ADT)

ADT is the standard of care for the treatment of metastatic prostate cancer and can be carried out by various approaches, including orchiectomy, chemical castration, antiandrogen therapy and/or combinations thereof. Chemical castration employs chemicals to reduce testosterone production in the testes thus preventing androgen stimulation of prostate cancer cells. Currently, the chemicals for castration include luteinizing hormone releasing hormone (LHRH) or gonadotropin releasing hormone (GnRH) agonists or antagonists (1, 5).

Typically, androgens are primarily produced in the testes and adrenal glands. Unexpectedly, about 50% of CR prostate cancer cells exhibit intracrine regulation, i.e., they can perform *de novo* testosterone biosynthesis from cholesterol (10, 14). Hence, a novel avenue for treating CR prostate cancer is to inhibit androgen biosynthesis in those cells. To enhance the efficacy of ADT, antiandrogens can be utilized in conjunction with chemical or surgical castration, which will further reduce androgen stimulation of prostate cancer cells by mitigating the ability of cancer cells to synthesize or utilize androgen signaling. One class of antiandrogen are the androgen biosynthesis inhibitors, such as abiraterone acetate. These agents abrogate the activity of CYP17A, an enzyme involved in two crucial steps of androgen biosynthesis; therefore, these compounds are effective treatment options

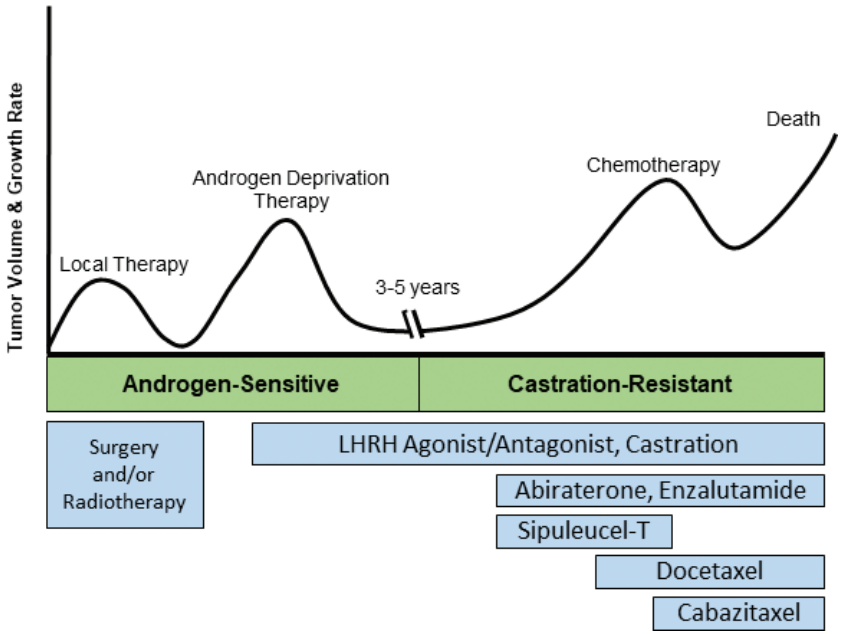

Figure 1. Prostate cancer progression and treatment options. Most prostate cancer cases are detected prior to its spread to other parts of the body. Surgery and radiotherapy can treat localized tumors. The standard-of-care treatment for metastatic prostate cancer is ADT. Nevertheless, most metastatic prostate cancer will relapse, i.e., the development of CR prostate cancer. The CR prostate cancer can be initially treated with antiandrogens such as enzalutamide, abiraterone or with immunotherapy agent such as Sipuleucel-T. Upon further progression of the disease, docetaxel and cabaitaxel can be used, in addition to abiraterone and enzalutamide, if the patient has not previously been treated with these agents. Unfortunately, these CR prostate cancer treatments will only prolong a patient's life by less than one year before they succumb to the disease (1–3).

for prostate cancer that is capable of intra-tumoral androgen biosynthesis. A phase III clinical trial (NCT00887198) in chemotherapy-naïve CR prostate cancer patients showed a 3.7 month increase in overall survival as well as increased time to initiation of chemotherapy and PSA progression upon abiraterone treatment (24). The phase III trial (NCT00638690) of abiraterone after progression on docetaxel prolonged the survival of patients by four months and increased progression-free survival (PFS) by two months (25). These two studies led to the FDA approval of abiraterone acetate in 2011 for treatment of CR prostate cancer in chemotherapy-naïve patients as well as upon docetaxel resistance.

Another class of antiandrogens is the AR blocker, an AR antagonist that prevents androgen receptors from nuclear translocation and DNA binding. AR blocker bicalutamide (Casodex) and second-generation agent enzalutamide (Xtandi) were FDA-approved in 1995 and 2012, respectively, for the treatment of prostate cancer. The phase III AFFIRM trial (NCT00974311) determined that enzalutamide prolongs the survival period by five months in CR prostate cancer patients, post-docetaxel treatment (26). In the PREVAIL phase III trial

TABLE 1 A list of completed clinical trials of combination treatments for CR prostate cancer

Clinical Trial	Primary Anticancer Agent	Secondary Anticancer Agent	Result	Reference
NCT00002874	ADT	Radiation	Reduced mortality and metastasis compared to ADT alone	Shipley et al. 2017 (39)
ISRCTN01534787	ADT	Radiation	Reduced 10-year mortality rate and PSA recurrence	Widmark et al. 2009 (36)
NCT00002633/ ISRCTN24991896	ADT	Radiation	Reduced mortality rate	Warde et al. 2011 (37)
GETUG-AFU 15 (NCT00104715)	ADT	Docetaxel	Increased Survival by 5.7 Months; Toxic to Patients	Gravis et al. 2013 (44)
CHAARTED (NCT00309985)	ADT	Docetaxel	Increased Survival by 13.6 Months Compared to ADT Alone	Sweeny et al. 2015 (45)
STAMPEDE (NCT00268476)	ADT	Docetaxel	Increased Survival by 10 Months; Toxic to Patients	James et al. 2016 (46)
STAMPEDE (NCT00268476)	ADT	Zolendronic Acid	No Survival Benefit	James et al. 2016 (46)
NCT01972217	Abiraterone	Olaparib	Increased survival by 5.6 months compared to abiraterone alone	Clarke et al. 2018 (50)
ERA 223 (NCT02043678)	Abiraterone	Radium 223	No Survival Benefit	Smith et al. 2019 (43)
	Abiraterone	Enzalutamide	No Survival Benefit	Efstathiou et al. 2020 (90)
NCT01807065	Sipuleucel-T	Radiation	Radiation does not affect delivery or effectiveness of Sipuleucel-T	Twardowski et al. 2017 (65)
	Sipuleucel-T	Docetaxel	Increases Survival by 10 Months	Petrylak 2007 (66)

Table continued on following page

TABLE 1 A list of completed clinical trials of combination treatments for CR prostate cancer (Continued)

Clinical Trial	Primary Anticancer Agent	Secondary Anticancer Agent	Result	Reference
NCT00861614	Ipilimumab	Radiation	No Survival Benefit	Kwon et al. 2014 (70)
NCT02484404	Durvalumab	Olaparib	PSA and radiographic response	Karzai et al. 2018 (71)
NCT00091364	Docetaxel	Thalidomide and Bevacizumab	Median Survival Time of 28.2 Months; Well-tolerated	Ning et al. 2010 (72)
MAINSAIL (NCT00988208)	Docetaxel	Lenalidomide	Reduced Overall Survival	Petrylak et al. 2015 (73)
NCT00110214	Docetaxel	Bevacizumab	No Overall Survival Benefit	Kelly et al. 2012 (74)
READY (NCT00744497)	Docetaxel	Dastinib	No Survival Benefit	Araujo et al. 2013 (80)
NCT01685125	Abiraterone	Dastinib	No Survival Benefit	Dorff et al. 2019 (81)
TRAPEZE (NCT00554918)	Docetaxel	Zolendronic Acid	Reduced Bone Metastasis, No Survival Benefit	James et al. 2016 (82)
SYNERGY (NCT01188187)	Docetaxel	Custirsen	No Survival Benefit	Chi et al. 2017 (83)
AFFINITY (NCT01578655)	Cabazitaxel	Custirsen	No Survival Benefit	Beer et al. 2017 (84)

TABLE 2 Ongoing clinical trials for combination therapies in prostate cancer

Clinical Trial	Primary Anticancer Agent	Secondary Anticancer Agent	Current Status	Phase
LACOG-0415 (NCT02867020)	Abiraterone	Apalutamide	Recruiting	2
LATITUDE	Abiraterone	ADT	Active, not recruiting	3
NCT03732820	Abiraterone	Olaparib	Recruiting	3
NCT00450463	ADT	PROSTVAC	Completed (No compiled results)	2
NCT01867333	ADT	PROSTVAC	Active, not recruiting	2
NCT02913196	Apalutamide	Abiraterone, Docetaxel	Recruiting	1
NCT01420250	Cabazitaxel	Radiation, ADT	Active, not recruiting	1
NCT02649855	Docetaxel	PROSTVAC-IF	Active, not recruiting	2
NCT01555242	Docetaxel	Aneustat	Completed (No compiled results)	1
NCT03834506	Docetaxel	Pembrolizumab	Recruiting	3
NCT02788773	Durvalumab	Tremelimumab (Anti-CTLA-4)	Active, not recruiting	2
NCT02207504	Enzalutamide	Crizotinib (TKI)	Active, not recruiting	1
NCT03834493	Enzalutamide	Pembrolizumab	Recruiting	3
NCT02280356	ERBT	Brachytherapy	Active, not recruiting	2
NCT01688492	Ipilimumab	Abiraterone	Active, not recruiting	1 and 2
NCT03488810	LHRH	Apalutamide, Radiation	Not yet recruiting	3
NCT03810105	Olaparib	Durvalumab	Recruiting	2

Table continued on following page

TABLE 2 Ongoing clinical trials for combination therapies in prostate cancer (Continued)

Clinical Trial	Primary Anticancer Agent	Secondary Anticancer Agent	Current Status	Phase
NCT02861573	Pembrolizumab	Various Therapeutics	Recruiting	1
NCT03910660	Pembrolizumab	BXCL701 (immune activator)	Recruiting	1 and 2
NCT03805594	Pembrolizumab	177Lu-PSMA-617 (Radioconjugated PSMA)	Recruiting	1
NCT04191096	Pembrolizumab	ADT, Enzalutamide	Recruiting	3
NCT03315871	PROSTVAC	MSB0011359C (anti-PD-L1 and TGF-β) CV301 (anti-CEA and Muc-1)	Recruiting	2
PEACE III (NCT02194842)	Radium 223	Enzalutamide	Recruiting	3
NCT03574571	Radium 223	Docetaxel	Recruiting	3
NCT03737370	Radium 223	Docetaxel	Recruiting	2
NCT02463799	Sipuleucel-T	Radium 223	Active, not recruiting	2

(NCT01212991), enzalutamide was shown to delay radiographic disease progression in chemotherapy-naïve patients with 65% of patients disease-free for 12 months compared to 14% with the placebo. This trial also showed an improved overall survival of two months with enzalutamide treatment (27). Interestingly, the STRIVE trial (NCT01664923) in CR prostate cancer patients showed that enzalutamide had a significantly higher PFS at 19.4 months compared to 5.7 months with bicalutamide, and increased time to PSA progression (28). These results have led to the popularity of enzalutamide over bicalutamide in treating prostate cancer in recent years. Recently, apalutamide (Erleada) was approved by the FDA for treatment of non-metastatic CR prostate cancer due to the success of the SPARTAN trials (NCT01946204) which demonstrated that apalutamide could prolong metastasis-free survival by over two years (29).

While ADT has been the gold standard for treating metastatic prostate cancer since 1941 (4), and is a life-long treatment, this therapy eventually fails. Therefore, ongoing studies are currently analyzing potential combination treatments to reduce the risk of recurrence after ADT or to treat CR prostate cancer.

Taxanes

Taxanes are anticancer agents that stabilize microtubules to prevent cell division and mitosis, and thus result in cell death of rapidly dividing tumor cells. Paclitaxel (Taxol) is the first and most common of these anti-microtubule agents for advanced cancer treatments (FDA approved in 1998). Docetaxel (Taxotere) is one of the few FDA-approved drugs for CR prostate cancer. In combination with prednisone, docetaxel has been shown to provide a survival benefit of 2.4 months compared with mitoxantrone. Cabazitaxel (Jevtana) is another member of the taxane family used to combat docetaxel resistance in several cancers; however, resistance to both taxanes can occur via upregulation of ABC1 transporter P-glycoprotein (30, 31). It should be noted that taxanes are highly toxic, often leading to severe side effects in patients (30). Hence, the development of more selective compounds continues.

Sipuleucel-T

Sipuleucel-T is an immunology product of peripheral blood mononuclear cells harvested by leukophoresis. The dendritic cells are co-cultured with PA2024, a recombinant fusion protein of prostatic acid phosphatase (PAcP) and granulocyte-macrophage colony-stimulating factor (GM-CSF) before being infused into the patient. PAcP is a prostate epithelia-specific differentiation antigen (11–13) expressed in about 95% of prostate cancers, while GM-CSF stimulates dendritic cell maturity and activation. The infusion of these autologous dendritic cells stimulates the patient's immune system, particularly antitumor T-cells, to target the cancer cells. This immunotherapy has been shown to provide a survival benefit of 4.5 months in patients with CR prostate cancer and is well tolerated by patients (32, 33). The IMPACT trial demonstrated that patients received the greatest effects from Sipuleucel-T when they had low PSA levels (34). Sipuleucel-T was approved by the FDA in 2010 as a first- or second-line therapy for the treatment of asymptomatic or minimally symptomatic metastatic CR prostate cancer before or after

docetaxel therapy (35). Currently, studies continue to expand the potential pool of immunological products that can be utilized for treating prostate cancer, including T cell activator ipilimumab, and anti-ErbB-2 antibodies trastuzumab and pertuzumab among others.

COMBINATION TREATMENTS FOR CASTRATION-RESISTANT PROSTATE CANCER

Prostate cancer often develops resistance to, and progress on, the various therapies discussed above. Combination therapies with current treatment strategies could effectively suppress the tumor and increase lifespan of patients. Most combinations utilize ADT or androgen deprived conditions, and combination therapies with radiation, chemotherapy, and immunotherapy show promise.

Combination of radiation with ADT

As the standard-of-care treatment of metastatic prostate cancer, ADT has more potential treatment combinations compared to other FDA-approved drugs for CR prostate cancer. An increasing number of patients with high-risk disease are treated with ADT and radiation therapy to prevent or delay the development of CR prostate cancer. The use of ADT before, during, and after radiation therapy is now highly encouraged for patients with intermediate- or high-risk disease. Overall survival, disease-free survival, distant metastasis-free survival, and biochemical-free survival rates all increase upon combination of ADT with radiotherapy compared to radiotherapy alone (36–39). The detailed synergistic mechanism of androgen suppression with local radiotherapy remains under investigation. One proposed mechanism is that AR suppression may lead to a downregulation of non-homologous end-joining (NHEJ) and further sensitization of prostate cancer cells to radiation (40). Nevertheless, it should be noted that the combination treatment of ADT and radiotherapy may cause the adverse increase of NE-like prostate cancer cell populations, therefore increasing the potential for resistance of CR prostate cancer to treatments (41). Patients can also be overtreated by the combination of ADT and radiotherapy. Hence, the optimal timing and duration of this combination should be further studied to reduce adverse effects (36–39). There is an emerging role of prostate radiotherapy in advanced and metastatic disease based upon the STAMPEDE trial (NCT00268476), wherein patients were randomized to receive radiation therapy to the prostate despite a diagnosis of metastatic disease (36–39). In that study, while it showed no overall survival benefits, patients with low volume metastatic disease were shown to have an overall survival advantage.

Radium-223 dichloride (radium-223, Xofigo) deserves attention. Radium-223 emits low levels of alpha particle radiation resulting in DNA double-strand breaks and cell death. It is also a “calcium mimetic”. The ALSYMPCA trial (the Alpharadin in Symptomatic Prostate Cancer Patients, NCT00699751) with Radium-223 alone in treating metastatic, CR prostate cancer showed a significant efficacy in overall survival (14.9 months vs. 11.3 months) in all patient subgroups with significantly fewer adverse events than placebo patients (42). Nevertheless, the ERA

223 trial, which combined abiraterone with Radium-223 found no improvement in skeletal event-free survival; instead, there was an increased occurrence of bone fractures with this combination (43). Hence, osteoprotective agents have been suggested and studies continue with combinations of Radium-223 with enzalutamide (PEACE III trial). The benefits of combination treatment of Radium-223 with additional therapies beyond ADT remain under further investigation for CR prostate cancer.

Combination of chemotherapy with ADT

ADT with chemotherapy is another potential combination treatment option to target both androgen-sensitive (AS) and androgen-independent (AI) prostate cancer cells. The GETUG-AFU 15 trial (NCT00104715) revealed that ADT with docetaxel increased survival by 5.7 months, however, this combination increases severe side effects as well as deaths due to the toxicity of docetaxel (44). The CHAARTED trial (NCT00309985) found a statistically significant increase in overall survival with this combination, extending a patient's life by 13.6 months longer than ADT alone as well as providing an 8.5 month increase in time to biochemical, symptomatic, and radiographic progression (45). The STAMPEDE trial (NCT00268476) confirmed that the combination of ADT and docetaxel provides a survival benefit of about 10 months compared to ADT alone as well as an increase in PFS seen in the CHAARTED trial, and confirmed the high toxicity found in the GETUG-AFU 15 trial. Additionally, the STAMPEDE trial showed that zoledronic acid (Zometa), an agent that slows osteoclast activity, had little effect on the survival of prostate cancer patients in combination with ADT (46). Short and long-term toxicities for docetaxel are real, and efforts are needed to find alternative agents or mitigate toxicity.

Extracellular signal-regulated kinase (ERK) inhibitors are a possible alternative to reduce taxane toxicity. ERK inhibitors can increase the potency of docetaxel on CR prostate cancer cells (47). Hence, ERK inhibitors can be employed with docetaxel under ADT, which will reduce the docetaxel dosage as well as its toxicity while achieving a similar therapeutic index (47). Future clinical trials of this new combination may shed more light on this subject.

Other chemotherapeutics for combination treatment of CR prostate cancer are Poly (ADP-ribose) polymerase (PARP) inhibitors. Two pre-clinical models showed synergistic anticancer effects of Olaparib and enzalutamide in androgen-sensitive and -independent cell lines and in xenograft models (48, 49). The combination of abiraterone and Olaparib initially in a phase II trial (NCT01972217) found a 5.6 month increase in PFS in metastatic CR prostate cancer patients compared to abiraterone alone (50). A Phase III trial (NCT03732820) for this combination has had significant outcomes, including extending survival in a biomarker selected population. Hence, both inhibitors Rucaparib and Olaparib have received FDA approval for treating metastatic CR prostate cancer with specific genetic alterations.

Combination of immunotherapy with ADT

Immunotherapy, namely cancer vaccines, represent another promising treatment to combine with ADT. One such option is a PSA-targeted poxviral vaccine,

PROSTVAC-IF, which was initially reported to reduce death rates by 44% and provide prostate cancer patients with an 8.5 month increase in survival alone (51). Nevertheless, further studies showed that PROSTVAC-IF alone did not effectively increase overall survival (52). Prostate cancer-specific immunotherapy is thus being explored as part of combination treatments. Currently, two phase III clinical trials are ongoing which analyze the survival effects of the combination of PROSTVAC-IF with ADT (NCT00450463, NCT01867333).

Other preclinical studies include the analysis of CAR-T cells targeted to Muc1, a glycoprotein that is often expressed on the surface of prostate cancer cells but not in non-cancerous tissues. Studies found that Muc1 CAR-T cells effectively reduce prostate cancer tumor growth in combination with the antiandrogen flutamide. The study further ensured that flutamide does not negatively affect CAR-T-Muc1 activity (53). Combination therapy can also be utilized through targeting prostate-specific membrane antigen (PSMA); Murga *et al.* (54) showed that an anti-PSMA antibody conjugated to anti-microtubule agent monomethyl auristatin E is effective against prostate cancer cells that express PSMA. The combination of this antibody-drug conjugate with enzalutamide or abiraterone resulted in the synergistic inhibition of prostate cancer growth, as the antiandrogens increased the expression of PSMA. Recent advances determined that insulin-like growth factor (IGF) also contributes to castration-resistance. Hence, targeting PSMA with the IGF-1/IGF-2 neutralizing antibody xenuzumab in combination with enzalutamide has been successful in inhibiting prostate cancer growth in preclinical models (55).

Combination of targeting androgen biosynthesis and ADT

Several studies have investigated combinations with androgen biosynthesis inhibitors beyond those traditionally utilized in ADT for CR prostate cancer treatment. Shutting down androgen signaling with concurrent abiraterone and enzalutamide treatment was found to be of no benefit (54). Alternately, while combining abiraterone and LHRH agonists could reduce tumor burden, it produced no change to patient outcomes. Unexpectedly, the study discovered the upregulation of glucocorticoid receptor (GR) in response to androgen blockage, suggesting a potential mechanism of resistance (56). Hamid *et al.* showed that the combination of dutasteride, a 5α-reductase inhibitor, and enzalutamide resulted in a synergistic inhibition of prostate cancer cell growth in culture (57).

Analyses of cohort studies revealed that the usage of statins (cholesterol-lowering drugs) correlated with reduced risk of several cancers, including prostate cancer and its advanced stage progression, as well as increases in survival rates. This could be due to the fact that cholesterol is the unique source of steroid biosynthesis, including testosterone, which prostate cancer cells rely on. Several studies have shown that the combination of ADT and statins reduces the risk of advanced prostate cancer and increases the survival rates of prostate cancer patients (58–60). The combination of statins and abiraterone exhibits an added effect of cell growth inhibition (61). Interestingly, a novel statin derivative simvastatin hydroxyl acid (SVA) appears to be more potent than its parent compound simvastatin toward CR prostate cancer cells, with minimal

toxicity (62). Further, SVA exhibits an added inhibitory effect on CR prostate cancer cells in cultures in combination with abiraterone acetate or docetaxel (personal observation). Due to promising *in vitro* studies, the potential clinical usage of SVA in combination with ADT for treating CR prostate cancer warrants further investigation.

Combination of radiation therapy with immunotherapy under ADT

Radiation was initially thought to be immunosuppressive, thus combining immunotherapy treatments with radiation was considered implausible. Nevertheless, radiation is not as detrimental to the immune system as initially thought and can even stimulate an immune response to a variety of cancers (63). Kwilas *et al*. first showed that there was evidence of synergy in the combination of immunotherapy and radiation (64). Subsequently, many studies have tailored this combination to their specific cancer of interest.

In the context of prostate cancer, a phase II trial (NCT01807065) analyzed the combination of sipuleucel-T and radiation in men with CR prostate cancer. It was shown that radiation therapy did not affect product parameters or delivery of sipuleucel-T therapy (65). Clinical trials of this combination are ongoing to determine if sipuleucel-T and radiation therapy provide a survival benefit to patients with CR prostate cancer.

Combination of chemotherapy with immunotherapy under ADT

A phase III trial of Sipuleucel-T combined with docetaxel has been conducted, in which it was found there was about a 10 month increase in overall survival when patients were treated with docetaxel several months after Sipuleucel-T treatment (66). In parallel, efforts are still ongoing to get FDA approval of PROSTVAC-IF for treatment of prostate cancer or CR prostate cancer. A phase III clinical trial that combines PROSTVAC-IF with docetaxel (NCT02649855) is underway.

Clinical trials with immune checkpoint inhibitors as a single agent have been unsuccessful (67). Therefore, studies have looked to combinations with these molecules. A phase I/II study in CR prostate cancer analyzed the effects of ipilimumab, a monoclonal antibody that blocks the binding of immunoregulatory molecule Cytotoxic T-lymphocyte Associated Protein 4 (CTLA-4) to its ligand to enhance T cell activation and proliferation (68). Trials showed the combination of ipilimumab and radiation therapy were well tolerated by patients, and had effective antitumor properties, including a 50% reduction in PSA levels and stable disease (69). Unfortunately, a phase III trial showed that this combination provided no survival advantage in patients with docetaxel-resistant prostate cancer (70). Interestingly, the anti-PD-L1 antibody durvalumab has had some success in the clinical setting. A small study by Karzai *et al.* determined that durvalumab with Olaparib was effective against CR prostate cancer with a high mutational burden in DNA damage response proteins (71). Many other studies with immunotherapies, including pembrolizumab and durvalumab, are currently in progress with combination therapies, including Olaparib, AKT inhibitors and others, for CR prostate cancer treatment.

Combinations with docetaxel under ADT

Treatment combinations with docetaxel have had mixed results on patient survival. For example, thalidomide in combination with anti-VEGF-A antibody bevacizumab and docetaxel effectively reduced PSA levels in a phase II clinical trial (NCT00091364) (72). A phase III trial found that the combination of lenalidomide with docetaxel reduced patient survival compared to docetaxel alone due to toxicity (73). While adding anti-angiogenic agent bevacizumab to this particular combination resulted in significant reduction in PSA and disease, a phase III trial (NCT00110214) of docetaxel with prednisone and bevacizumab showed that there was no difference in overall survival in treatments with or without bevacizmab (74).

Efforts have continued on developing new compounds and combinations. As for pre-clinical models, the combination of fatty acid binding protein 5 (FABP5) inhibitors and docetaxel or cabazitaxel shows synergistic cytotoxic effects *in vitro* and *in vivo* (75). Similarly, docetaxel nanoparticles in combination with the receptor activator of nuclear factor κB ligand (RANKL) monoclonal antibody, denosumab, led to an increase in survival and reduction in tumor burden and bone metastasis in prostate cancer xenograft animal model (76). Combination of docetaxel with anti-microtubule agent mebendazole was found to be effective against prostate cancer; further analysis showed enhanced anti-tumor activity without toxicity (77).

Novel small molecule inhibitors as single agents and their combinations

Small molecule inhibitors are potentially useful agents against prostate cancer, either as single agents or in combination with ADT. Phosphatase and tensin homolog (PTEN) loss is common in advanced prostate cancer, thus targeting the AKT/mammalian target of rapamycin (mTOR) pathway—a PTEN-regulating pathway—could represent a viable therapeutic option (78). Preclinical models showed that inhibition of phosphoinositide 3-kinase (PI3K) or AKT with small molecules AZD8186 or AZD5363, respectively, in combination with ADT resulted in enhanced growth suppression of xenograft prostate cancer tumors (79). Nevertheless, AKT inhibitors can cause an elevation of PSA level (62). Hence, alternate biomarker(s) for this treatment should be developed.

Small molecule inhibitors have also been combined with docetaxel with mixed results. Due to the frequent alterations in kinase signaling pathways upon progression to the CR phenotype (8, 47), inhibition of tyrosine kinases and corresponding downstream molecules was attempted as a treatment for CR prostate cancer. While Phase I/II trials (NCT00439270) showed that dasatinib (Sprycel), a Src and BCR-ABL tyrosine kinase inhibitor (TKI), in combination with docetaxel was well tolerated by patients, the phase III READY trial (NCT00744497) revealed no improvement in patient survival (80). The combination of dasatinib and abiraterone also did not show any benefit to patients (81). Phase III TRAPEZE trial (NCT00554918) combining docetaxel with strontium-89, zoledronic acid, or both showed significantly reduced bone metastasis while having no effect on overall patient survival upon treatment with

zoledronic acid and docetaxel (82). The SYNERGY trial (NCT01188187) demonstrated that the combination of docetaxel and custirsen, an antisense oligonucleotide that inhibits production of resistance-associated chaperone protein Clusterin, also does not improve overall patient survival (83). The AFFINITY trial (NCT01578655) also resulted in no improvement for patient survival with a combination of custirsen and cabazitaxel (84).

An interesting small molecule is Aneustat (OMN54), a multivalent botanical drug undergoing a phase I clinical trial (NCT01555242) for advanced cancers, primarily lymphomas. Pre-clinical studies revealed that docetaxel and Aneustat treatment reduced the growth of prostate cancer LNCaP C4–2 cell line and LTL-313H prostate cancer tissue mouse xenografts with potential synergistic effects via inhibition of AR, AKT phosphorylation and Bcl-2 expression (85). Hence, it is proposed that combination of docetaxel with Aneustat could further extend the life of prostate cancer patients. Meanwhile, early *ex vivo* studies showed that the combination of docetaxel and dopamine D2 receptor agonist bromocriptine effectively reduced tumor growth and bone metastasis in prostate cancer xenograft models (86), a potential novel combination for prostate cancer treatment.

Development of more novel compounds for CR prostate cancer treatment is equally important. For example, statin derivative SVA, imidazopyridine derivatives, and pregnene analogs (87–89) have been shown to be effective against CR prostate cancer cells under androgen-reduced conditions. It is imperative to continue the efforts on investigating their utilities in CR prostate cancer therapy.

CONCLUSION

In summary, while no single agent or agent combinations are shown to cure CR prostate cancer patients, significant progress continues to be made and patients are living longer with advanced prostate cancer. We propose that the next immediate step in the management of metastatic prostate cancer is to make CR prostate cancer a chronic disease, while improving the patient's quality of life. Together, these will accomplish our immediate goal of reducing the lethality of prostate cancer. While the advancement of current combinations is important, it is also imperative to develop novel compounds that can target both the adenocarcinoma and the neuroendocrine prostate cancer cell populations, while sparing normal cells from cytotoxicity.

Acknowledgement: This work was supported in part by the National Institute of Health [R03 CA230950, R01 CA233664, F32 CA250129], Department of Defense PCRP Grants [PC121645 and PC141559], the University of Nebraska Medical Center Graduate Studies Fellowship, the Fred and Pamela Buffett Cancer Center Support Grant [P30 CA036727], the UNMC Cancer Biology Training Grant [T32CA009476], and the UNMC Purdue Pharma Scholars Award. The work is also supported in part by the University of Colorado Training Program in Cancer Biology [T32CA190216–04].

Conflict of Interest: The authors declare no potential conflicts of interest with respect to research, authorship and/or publication of this chapter.

REFERENCES

1. Siegel RL, Miller KD, Jemal A. Cancer statistics, 2020. CA Cancer J Clin. 2020;70(1):7–30. https://doi.org/10.3322/caac.21590
2. Mohler JL, Armstrong AJ, Bahnson RR, D'Amico AV, Davis BJ, Eastham JA, et al. Prostate Cancer, Version 1.2016. J Natl Compr Canc Netw. 2016;14(1):19–30.
3. McKay RR, Feng FY, Wang AY, Wallis CJD, Moses KA. Recent Advances in the Management of High-Risk Localized Prostate Cancer: Local Therapy, Systemic Therapy, and Biomarkers to Guide Treatment Decisions. Am Soc Clin Oncol Educ Book. 2020;40:1–12. https://doi.org/10.1200/EDBK_279459
4. Huggins C, Clark PJ. Quantitative Studies of Prostatic Secretion : Ii. The Effect of Castration and of Estrogen Injection on the Normal and on the Hyperplastic Prostate Glands of Dogs. J Exp Med. 1940;72(6):747–62. https://doi.org/10.1084/jem.72.6.747
5. Liu J, Geller J, Albert J, Kirshner M. Acute effects of testicular and adrenal cortical blockade on protein synthesis and dihydrotestosterone content of human prostate tissue. J Clin Endocrinol Metab. 1985;61(1):129–33. https://doi.org/10.1210/jcem-61-1-129
6. Taplin ME, Bubley GJ, Shuster TD, Frantz ME, Spooner AE, Ogata GK, et al. Mutation of the androgen-receptor gene in metastatic androgen-independent prostate cancer. N Engl J Med. 1995;332(21):1393–8. https://doi.org/10.1056/NEJM199505253322101
7. Craft N, Shostak Y, Carey M, Sawyers CL. A mechanism for hormone-independent prostate cancer through modulation of androgen receptor signaling by the HER-2/neu tyrosine kinase. Nat Med. 1999;5(3):280–5. https://doi.org/10.1038/6495
8. Lee MS, Igawa T, Yuan TC, Zhang XQ, Lin FF, Lin MF. ErbB-2 signaling is involved in regulating PSA secretion in androgen-independent human prostate cancer LNCaP C-81 cells. Oncogene. 2003;22(5):781–96. https://doi.org/10.1038/sj.onc.1206066
9. Stanbrough M, Bubley GJ, Ross K, Golub TR, Rubin MA, Penning TM, et al. Increased expression of genes converting adrenal androgens to testosterone in androgen-independent prostate cancer. Cancer Res. 2006;66(5):2815–25. https://doi.org/10.1158/0008-5472.CAN-05-4000
10. Dillard PR, Lin MF, Khan SA. Androgen-independent prostate cancer cells acquire the complete steroidogenic potential of synthesizing testosterone from cholesterol. Mol Cell Endocrinol. 2008;295(1–2):115–20. https://doi.org/10.1016/j.mce.2008.08.013
11. Muniyan S, Chen SJ, Lin FF, Wang Z, Mehta PP, Batra SK, et al. ErbB-2 signaling plays a critical role in regulating androgen-sensitive and castration-resistant androgen receptor-positive prostate cancer cells. Cell Signal. 2015;27(11):2261–71. https://doi.org/10.1016/j.cellsig.2015.08.002
12. Miller DR, Ingersoll MA, Lin MF. ErbB-2 signaling in advanced prostate cancer progression and potential therapy. Endocr Relat Cancer. 2019;26(4):R195-R209. https://doi.org/10.1530/ERC-19-0009
13. Lin MF, Lee MS, Garcia-Arenas R, Lin FF. Differential responsiveness of prostatic acid phosphatase and prostate-specific antigen mRNA to androgen in prostate cancer cells. Cell Biol Int. 2000;24(10):681–9. https://doi.org/10.1006/cbir.2000.0433
14. Debes JD, Tindall DJ. Mechanisms of androgen-refractory prostate cancer. N Engl J Med. 2004;351(15):1488–90. https://doi.org/10.1056/NEJMp048178
15. Bonkhoff H. Neuroendocrine differentiation in human prostate cancer. Morphogenesis, proliferation and androgen receptor status. Ann Oncol. 2001;12 Suppl 2:S141–4. https://doi.org/10.1093/annonc/12.suppl_2.S141
16. Yuan TC, Veeramani S, Lin FF, Kondrikou D, Zelivianski S, Igawa T, et al. Androgen deprivation induces human prostate epithelial neuroendocrine differentiation of androgen-sensitive LNCaP cells. Endocr Relat Cancer. 2006;13(1):151–67. https://doi.org/10.1677/erc.1.01043

17. Yuan TC, Veeramani S, Lin MF. Neuroendocrine-like prostate cancer cells: neuroendocrine transdifferentiation of prostate adenocarcinoma cells. Endocr Relat Cancer. 2007;14(3):531–47. https://doi.org/10.1677/ERC-07-0061
18. Filson CP, Marks LS, Litwin MS. Expectant management for men with early stage prostate cancer. CA Cancer J Clin. 2015;65(4):265–82. https://doi.org/10.3322/caac.21278
19. Smith GD, Pickles T, Crook J, Martin AG, Vigneault E, Cury FL, et al. Brachytherapy improves biochemical failure-free survival in low- and intermediate-risk prostate cancer compared with conventionally fractionated external beam radiation therapy: a propensity score matched analysis. Int J Radiat Oncol Biol Phys. 2015;91(3):505–16. https://doi.org/10.1016/j.ijrobp.2014.11.018
20. Goy BW, Burchette R, Soper MS, Chang T, Cosmatos HA. Ten-Year Treatment Outcomes of Radical Prostatectomy Vs External Beam Radiation Therapy Vs Brachytherapy for 1503 Patients With Intermediate-risk Prostate Cancer. Urology. 2020;136:180–9. https://doi.org/10.1016/j.urology.2019.09.040
21. Bach C, Pisipati S, Daneshwar D, Wright M, Rowe E, Gillatt D, et al. The status of surgery in the management of high-risk prostate cancer. Nat Rev Urol. 2014;11(6):342–51. https://doi.org/10.1038/nrurol.2014.100
22. Bill-Axelson A, Holmberg L, Ruutu M, Garmo H, Stark JR, Busch C, et al. Radical prostatectomy versus watchful waiting in early prostate cancer. N Engl J Med. 2011;364(18):1708–17. https://doi.org/10.1056/NEJMoa1011967
23. Hamdy FC, Donovan JL, Lane JA, Mason M, Metcalfe C, Holding P, et al. 10-Year Outcomes after Monitoring, Surgery, or Radiotherapy for Localized Prostate Cancer. N Engl J Med. 2016;375(15):1415–24. https://doi.org/10.1056/NEJMoa1606220
24. Ryan CJ, Smith MR, de Bono JS, Molina A, Logothetis CJ, de Souza P, et al. Abiraterone in metastatic prostate cancer without previous chemotherapy. N Engl J Med. 2013;368(2):138–48. https://doi.org/10.1056/NEJMoa1209096
25. de Bono JS, Logothetis CJ, Molina A, Fizazi K, North S, Chu L, et al. Abiraterone and increased survival in metastatic prostate cancer. N Engl J Med. 2011;364(21):1995–2005. https://doi.org/10.1056/NEJMoa1014618
26. Scher HI, Fizazi K, Saad F, Taplin ME, Sternberg CN, Miller K, et al. Increased survival with enzalutamide in prostate cancer after chemotherapy. N Engl J Med. 2012;367(13):1187–97. https://doi.org/10.1056/NEJMoa1207506
27. Beer TM, Armstrong AJ, Rathkopf DE, Loriot Y, Sternberg CN, Higano CS, et al. Enzalutamide in metastatic prostate cancer before chemotherapy. N Engl J Med. 2014;371(5):424–33. https://doi.org/10.1056/NEJMoa1405095
28. Penson DF, Armstrong AJ, Concepcion R, Agarwal N, Olsson C, Karsh L, et al. Enzalutamide Versus Bicalutamide in Castration-Resistant Prostate Cancer: The STRIVE Trial. J Clin Oncol. 2016;34(18):2098–106. https://doi.org/10.1200/JCO.2015.64.9285
29. Smith MR, Saad F, Chowdhury S, Oudard S, Hadaschik BA, Graff JN, et al. Apalutamide Treatment and Metastasis-free Survival in Prostate Cancer. N Engl J Med. 2018;378(15):1408–18. https://doi.org/10.1056/NEJMoa1715546
30. de Bono JS, Oudard S, Ozguroglu M, Hansen S, Machiels JP, Kocak I, et al. Prednisone plus cabazitaxel or mitoxantrone for metastatic castration-resistant prostate cancer progressing after docetaxel treatment: a randomised open-label trial. Lancet. 2010;376(9747):1147–54. https://doi.org/10.1016/S0140-6736(10)61389-X
31. Lombard AP, Liu C, Armstrong CM, Cucchiara V, Gu X, Lou W, et al. ABCB1 Mediates Cabazitaxel-Docetaxel Cross-Resistance in Advanced Prostate Cancer. Mol Cancer Ther. 2017;16(10):2257–66. https://doi.org/10.1158/1535-7163.MCT-17-0179
32. Small EJ, Schellhammer PF, Higano CS, Redfern CH, Nemunaitis JJ, Valone FH, et al. Placebo-controlled phase III trial of immunologic therapy with sipuleucel-T (APC8015) in patients with metastatic, asymptomatic hormone refractory prostate cancer. J Clin Oncol. 2006;24(19):3089–94. https://doi.org/10.1200/JCO.2005.04.5252
33. Kantoff PW, Higano CS, Shore ND, Berger ER, Small EJ, Penson DF, et al. Sipuleucel-T immunotherapy for castration-resistant prostate cancer. N Engl J Med. 2010;363(5):411–22. https://doi.org/10.1056/NEJMoa1001294

34. Schellhammer PF, Chodak G, Whitmore JB, Sims R, Frohlich MW, Kantoff PW. Lower baseline prostate-specific antigen is associated with a greater overall survival benefit from sipuleucel-T in the Immunotherapy for Prostate Adenocarcinoma Treatment (IMPACT) trial. Urology. 2013;81(6): 1297–302. https://doi.org/10.1016/j.urology.2013.01.061
35. Shore ND, Mantz CA, Dosoretz DE, Fernandez E, Myslicki FA, McCoy C, et al. Building on sipuleucel-T for immunologic treatment of castration-resistant prostate cancer. Cancer Control. 2013;20(1): 7–16. https://doi.org/10.1177/107327481302000103
36. Widmark A, Klepp O, Solberg A, Damber JE, Angelsen A, Fransson P, et al. Endocrine treatment, with or without radiotherapy, in locally advanced prostate cancer (SPCG-7/SFUO-3): an open randomised phase III trial. Lancet. 2009;373(9660):301–8. https://doi.org/10.1016/S0140-6736(08)61815-2
37. Warde P, Mason M, Ding K, Kirkbride P, Brundage M, Cowan R, et al. Combined androgen deprivation therapy and radiation therapy for locally advanced prostate cancer: a randomised, phase 3 trial. Lancet. 2011;378(9809):2104–11. https://doi.org/10.1016/S0140-6736(11)61095-7
38. Bolla M, Verry C, Long JA. High-risk prostate cancer: combination of high-dose, high-precision radiotherapy and androgen deprivation therapy. Curr Opin Urol. 2013;23(4):349–54. https://doi.org/10.1097/MOU.0b013e328361ebfd
39. Shipley WU, Seiferheld W, Lukka HR, Major PP, Heney NM, Grignon DJ, et al. Radiation with or without Antiandrogen Therapy in Recurrent Prostate Cancer. N Engl J Med. 2017;376(5):417-28. https://doi.org/10.1056/NEJMoa1607529
40. Zhang W, Liao CY, Chtatou H, Incrocci L, van Gent DC, van Weerden WM, et al. Apalutamide Sensitizes Prostate Cancer to Ionizing Radiation via Inhibition of Non-Homologous End-Joining DNA Repair. Cancers (Basel). 2019;11(10). https://doi.org/10.3390/cancers11101593
41. Deng X, Elzey BD, Poulson JM, Morrison WB, Ko SC, Hahn NM, et al. Ionizing radiation induces neuroendocrine differentiation of prostate cancer cells in vitro, in vivo and in prostate cancer patients. Am J Cancer Res. 2011;1(7):834–44.
42. Parker C, Nilsson S, Heinrich D, Helle SI, O'Sullivan JM, Fossa SD, et al. Alpha emitter radium-223 and survival in metastatic prostate cancer. N Engl J Med. 2013;369(3):213–23. https://doi.org/10.1056/NEJMoa1213755
43. Smith M, Parker C, Saad F, Miller K, Tombal B, Ng QS, et al. Addition of radium-223 to abiraterone acetate and prednisone or prednisolone in patients with castration-resistant prostate cancer and bone metastases (ERA 223): a randomised, double-blind, placebo-controlled, phase 3 trial. Lancet Oncol. 2019;20(3):408–19. https://doi.org/10.1016/S1470-2045(18)30860-X
44. Gravis G, Fizazi K, Joly F, Oudard S, Priou F, Esterni B, et al. Androgen-deprivation therapy alone or with docetaxel in non-castrate metastatic prostate cancer (GETUG-AFU 15): a randomised, open-label, phase 3 trial. Lancet Oncol. 2013;14(2):149–58. https://doi.org/10.1016/S1470-2045(12)70560-0
45. Sweeney CJ, Chen YH, Carducci M, Liu G, Jarrard DF, Eisenberger M, et al. Chemohormonal Therapy in Metastatic Hormone-Sensitive Prostate Cancer. N Engl J Med. 2015;373(8):737–46. https://doi.org/10.1056/NEJMoa1503747
46. James ND, Sydes MR, Clarke NW, Mason MD, Dearnaley DP, Spears MR, et al. Addition of docetaxel, zoledronic acid, or both to first-line long-term hormone therapy in prostate cancer (STAMPEDE): survival results from an adaptive, multiarm, multistage, platform randomised controlled trial. Lancet. 2016;387(10024):1163–77. https://doi.org/10.1016/S0140-6736(15)01037-5
47. Zelivianski S, Spellman M, Kellerman M, Kakitelashvilli V, Zhou XW, Lugo E, et al. ERK inhibitor PD98059 enhances docetaxel-induced apoptosis of androgen-independent human prostate cancer cells. Int J Cancer. 2003;107(3):478–85. https://doi.org/10.1002/ijc.11413
48. Asim M, Tarish F, Zecchini HI, Sanjiv K, Gelali E, Massie CE, et al. Synthetic lethality between androgen receptor signalling and the PARP pathway in prostate cancer. Nat Commun. 2017;8(1):374. https://doi.org/10.1038/s41467-017-00393-y
49. Li L, Karanika S, Yang G, Wang J, Park S, Broom BM, et al. Androgen receptor inhibitor-induced "BRCAness" and PARP inhibition are synthetically lethal for castration-resistant prostate cancer. Sci Signal. 2017;10(480). https://doi.org/10.1126/scisignal.aam7479
50. Clarke N, Wiechno P, Alekseev B, Sala N, Jones R, Kocak I, et al. Olaparib combined with abiraterone in patients with metastatic castration-resistant prostate cancer: a randomised, double-blind,

placebo-controlled, phase 2 trial. Lancet Oncol. 2018;19(7):975–86. https://doi.org/10.1016/S1470-2045(18)30365-6
51. Kantoff PW, Schuetz TJ, Blumenstein BA, Glode LM, Bilhartz DL, Wyand M, et al. Overall survival analysis of a phase II randomized controlled trial of a Poxviral-based PSA-targeted immunotherapy in metastatic castration-resistant prostate cancer. J Clin Oncol. 2010;28(7):1099–105. https://doi.org/10.1200/JCO.2009.25.0597
52. Gulley JL, Borre M, Vogelzang NJ, Ng S, Agarwal N, Parker CC, et al. Phase III Trial of PROSTVAC in Asymptomatic or Minimally Symptomatic Metastatic Castration-Resistant Prostate Cancer. J Clin Oncol. 2019;37(13):1051–61. https://doi.org/10.1200/JCO.18.02031
53. Sanchez C, Chan R, Bajgain P, Rambally S, Palapattu G, Mims M, et al. Combining T-cell immunotherapy and anti-androgen therapy for prostate cancer. Prostate Cancer Prostatic Dis. 2013;16(2):123–31, S1. https://doi.org/10.1038/pcan.2012.49
54. Murga JD, Moorji SM, Han AQ, Magargal WW, DiPippo VA, Olson WC. Synergistic co-targeting of prostate-specific membrane antigen and androgen receptor in prostate cancer. Prostate. 2015;75(3):242–54. https://doi.org/10.1002/pros.22910
55. Weyer-Czernilofsky U, Hofmann MH, Friedbichler K, Baumgartinger R, Adam PJ, Solca F, et al. Antitumor Activity of the IGF-1/IGF-2-Neutralizing Antibody Xentuzumab (BI 836845) in Combination with Enzalutamide in Prostate Cancer Models. Mol Cancer Ther. 2020;19(4):1059–69. https://doi.org/10.1158/1535-7163.MCT-19-0378
56. Efstathiou E, Davis JW, Pisters L, Li W, Wen S, McMullin RP, et al. Clinical and Biological Characterisation of Localised High-risk Prostate Cancer: Results of a Randomised Preoperative Study of a Luteinising Hormone-releasing Hormone Agonist with or Without Abiraterone Acetate plus Prednisone. Eur Urol. 2019;76(4):418–24. https://doi.org/10.1016/j.eururo.2019.05.010
57. Hamid AR, Verhaegh GW, Smit FP, van Rijt-van de Westerlo C, Armandari I, Brandt A, et al. Dutasteride and enzalutamide synergistically suppress prostate tumor cell proliferation. J Urol. 2015;193(3):1023–9. https://doi.org/10.1016/j.juro.2014.09.021
58. Jacobs EJ, Rodriguez C, Bain EB, Wang Y, Thun MJ, Calle EE. Cholesterol-lowering drugs and advanced prostate cancer incidence in a large U.S. cohort. Cancer Epidemiol Biomarkers Prev. 2007;16(11):2213–7. https://doi.org/10.1158/1055-9965.EPI-07-0448
59. Harshman LC, Werner L, Tripathi A, Wang X, Maughan BL, Antonarakis ES, et al. The impact of statin use on the efficacy of abiraterone acetate in patients with castration-resistant prostate cancer. Prostate. 2017;77(13):1303–11. https://doi.org/10.1002/pros.23390
60. Di Lorenzo G, Sonpavde G, Pond G, Lucarelli G, Rossetti S, Facchini G, et al. Statin Use and Survival in Patients with Metastatic Castration-resistant Prostate Cancer Treated with Abiraterone Acetate. Eur Urol Focus. 2018;4(6):874–9. https://doi.org/10.1016/j.euf.2017.03.015
61. Miller DR, Ingersoll MA, Chou YW, Wakefield CB, Tu Y, Lin FF, et al. Anti-Androgen Abiraterone Acetate Improves the Therapeutic Efficacy of Statins on Castration-Resistant Prostate Cancer Cells. J Oncol Res Ther. 2017;3(5).
62. Ingersoll MA, Miller DR, Martinez O, Wakefield CB, Hsieh KC, Simha MV, et al. Statin derivatives as therapeutic agents for castration-resistant prostate cancer. Cancer Lett. 2016;383(1):94–105. https://doi.org/10.1016/j.canlet.2016.09.008
63. Chakraborty M, Abrams SI, Coleman CN, Camphausen K, Schlom J, Hodge JW. External beam radiation of tumors alters phenotype of tumor cells to render them susceptible to vaccine-mediated T-cell killing. Cancer Res. 2004;64(12):4328–37. https://doi.org/10.1158/0008-5472.CAN-04-0073
64. Kwilas AR, Donahue RN, Bernstein MB, Hodge JW. In the field: exploiting the untapped potential of immunogenic modulation by radiation in combination with immunotherapy for the treatment of cancer. Front Oncol. 2012;2:104. https://doi.org/10.3389/fonc.2012.00104
65. Twardowski P, Wong JYC, Pal SK, Maughan BL, Frankel PH, Franklin K, et al. Randomized phase II trial of sipuleucel-T immunotherapy preceded by sensitizing radiation therapy and sipuleucel-T alone in patients with metastatic castrate resistant prostate cancer. Cancer Treat Res Commun. 2019;19:100116. https://doi.org/10.1016/j.ctarc.2018.100116
66. Petrylak DP. New paradigms for advanced prostate cancer. Rev Urol. 2007;9 Suppl 2:S3-S12.
67. Beer TM, Kwon ED, Drake CG, Fizazi K, Logothetis C, Gravis G, et al. Randomized, Double-Blind, Phase III Trial of Ipilimumab Versus Placebo in Asymptomatic or Minimally Symptomatic

Patients With Metastatic Chemotherapy-Naive Castration-Resistant Prostate Cancer. J Clin Oncol. 2017;35(1):40–7. https://doi.org/10.1200/JCO.2016.69.1584
68. Maker AV, Phan GQ, Attia P, Yang JC, Sherry RM, Topalian SL, et al. Tumor regression and autoimmunity in patients treated with cytotoxic T lymphocyte-associated antigen 4 blockade and interleukin 2: a phase I/II study. Ann Surg Oncol. 2005;12(12):1005–16. https://doi.org/10.1245/ASO.2005.03.536
69. Slovin SF, Higano CS, Hamid O, Tejwani S, Harzstark A, Alumkal JJ, et al. Ipilimumab alone or in combination with radiotherapy in metastatic castration-resistant prostate cancer: results from an open-label, multicenter phase I/II study. Ann Oncol. 2013;24(7):1813–21. https://doi.org/10.1093/annonc/mdt107
70. Kwon ED, Drake CG, Scher HI, Fizazi K, Bossi A, van den Eertwegh AJ, et al. Ipilimumab versus placebo after radiotherapy in patients with metastatic castration-resistant prostate cancer that had progressed after docetaxel chemotherapy (CA184–043): a multicentre, randomised, double-blind, phase 3 trial. Lancet Oncol. 2014;15(7):700–12. https://doi.org/10.1016/S1470-2045(14)70189-5
71. Karzai F, VanderWeele D, Madan RA, Owens H, Cordes LM, Hankin A, et al. Activity of durvalumab plus olaparib in metastatic castration-resistant prostate cancer in men with and without DNA damage repair mutations. J Immunother Cancer. 2018;6(1):141. https://doi.org/10.1186/s40425-018-0463-2
72. Ning YM, Gulley JL, Arlen PM, Woo S, Steinberg SM, Wright JJ, et al. Phase II trial of bevacizumab, thalidomide, docetaxel, and prednisone in patients with metastatic castration-resistant prostate cancer. J Clin Oncol. 2010;28(12):2070–6. https://doi.org/10.1200/JCO.2009.25.4524
73. Petrylak DP, Vogelzang NJ, Budnik N, Wiechno PJ, Sternberg CN, Doner K, et al. Docetaxel and prednisone with or without lenalidomide in chemotherapy-naive patients with metastatic castration-resistant prostate cancer (MAINSAIL): a randomised, double-blind, placebo-controlled phase 3 trial. Lancet Oncol. 2015;16(4):417–25. https://doi.org/10.1016/S1470-2045(15)70025-2
74. Kelly WK, Halabi S, Carducci M, George D, Mahoney JF, Stadler WM, et al. 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. J Clin Oncol. 2012;30(13):1534–40. https://doi.org/10.1200/JCO.2011.39.4767
75. Carbonetti G, Converso C, Clement T, Wang C, Trotman LC, Ojima I, et al. Docetaxel/cabazitaxel and fatty acid binding protein 5 inhibitors produce synergistic inhibition of prostate cancer growth. Prostate. 2020;80(1):88–98. https://doi.org/10.1002/pros.23921
76. Vijayaraghavalu S, Gao Y, Rahman MT, Rozic R, Sharifi N, Midura RJ, et al. Synergistic combination treatment to break cross talk between cancer cells and bone cells to inhibit progression of bone metastasis. Biomaterials. 2020;227:119558. https://doi.org/10.1016/j.biomaterials.2019.119558
77. Rushworth LK, Hewit K, Munnings-Tomes S, Somani S, James D, Shanks E, et al. Repurposing screen identifies mebendazole as a clinical candidate to synergise with docetaxel for prostate cancer treatment. Br J Cancer. 2020;122(4):517–27. https://doi.org/10.1038/s41416-019-0681-5
78. Zhang W, Zhu J, Efferson CL, Ware C, Tammam J, Angagaw M, et al. Inhibition of tumor growth progression by antiandrogens and mTOR inhibitor in a Pten-deficient mouse model of prostate cancer. Cancer Res. 2009;69(18):7466–72. https://doi.org/10.1158/0008-5472.CAN-08-4385
79. Marques RB, Aghai A, de Ridder CMA, Stuurman D, Hoeben S, Boer A, et al. High Efficacy of Combination Therapy Using PI3K/AKT Inhibitors with Androgen Deprivation in Prostate Cancer Preclinical Models. Eur Urol. 2015;67(6):1177–85. https://doi.org/10.1016/j.eururo.2014.08.053
80. Araujo JC, Trudel GC, Saad F, Armstrong AJ, Yu EY, Bellmunt J, et al. Docetaxel and dasatinib or placebo in men with metastatic castration-resistant prostate cancer (READY): a randomised, double-blind phase 3 trial. Lancet Oncol. 2013;14(13):1307–16. https://doi.org/10.1016/S1470-2045(13)70479-0
81. Dorff TB, Quinn DI, Pinski JK, Goldkorn A, Sadeghi S, Tsao-Wei D, et al. Randomized Phase II Trial of Abiraterone Alone or With Dasatinib in Men With Metastatic Castration-resistant Prostate Cancer (mCRPC). Clin Genitourin Cancer. 2019;17(4):241–7 e1. https://doi.org/10.1016/j.clgc.2019.02.010
82. James ND, Pirrie SJ, Pope AM, Barton D, Andronis L, Goranitis I, et al. Clinical Outcomes and Survival Following Treatment of Metastatic Castrate-Refractory Prostate Cancer With Docetaxel Alone or With Strontium-89, Zoledronic Acid, or Both: The TRAPEZE Randomized Clinical Trial. JAMA Oncol. 2016;2(4):493–9. https://doi.org/10.1001/jamaoncol.2015.5570

83. Chi KN, Higano CS, Blumenstein B, Ferrero JM, Reeves J, Feyerabend S, et al. Custirsen in combination with docetaxel and prednisone for patients with metastatic castration-resistant prostate cancer (SYNERGY trial): a phase 3, multicentre, open-label, randomised trial. Lancet Oncol. 2017;18(4): 473–85. https://doi.org/10.1016/S1470-2045(17)30168-7
84. Beer TM, Hotte SJ, Saad F, Alekseev B, Matveev V, Flechon A, et al. Custirsen (OGX-011) combined with cabazitaxel and prednisone versus cabazitaxel and prednisone alone in patients with metastatic castration-resistant prostate cancer previously treated with docetaxel (AFFINITY): a randomised, open-label, international, phase 3 trial. Lancet Oncol. 2017;18(11):1532–42. https://doi.org/10.1016/S1470-2045(17)30605-8
85. Qu S, Wang K, Xue H, Wang Y, Wu R, Liu C, et al. Enhanced anticancer activity of a combination of docetaxel and Aneustat (OMN54) in a patient-derived, advanced prostate cancer tissue xenograft model. Mol Oncol. 2014;8(2):311–22. https://doi.org/10.1016/j.molonc.2013.12.004
86. Yang Y, Mamouni K, Li X, Chen Y, Kavuri S, Du Y, et al. Repositioning Dopamine D2 Receptor Agonist Bromocriptine to Enhance Docetaxel Chemotherapy and Treat Bone Metastatic Prostate Cancer. Mol Cancer Ther. 2018;17(9):1859–70. https://doi.org/10.1158/1535-7163.MCT-17-1176
87. Miller DR, Tzeng CC, Farmer T, Keller ET, Caplan S, Chen YS, et al. Novel CIL-102 derivatives as potential therapeutic agents for docetaxel-resistant prostate cancer. Cancer Lett. 2018;436:96–108. https://doi.org/10.1016/j.canlet.2018.07.039
88. Ingersoll MA, Lyons AS, Muniyan S, D'Cunha N, Robinson T, Hoelting K, et al. Novel Imidazopyridine Derivatives Possess Anti-Tumor Effect on Human Castration-Resistant Prostate Cancer Cells. PLoS One. 2015;10(6):e0131811. https://doi.org/10.1371/journal.pone.0131811
89. Abdul-Rida NA, Farhan AM, Al-Masoudi NA, Saeed BA, Miller D, Lin MF. A novel pregnene analogs: synthesis, cytotoxicity on prostate cancer of PC-3 and LNCPa-AI cells and in silico molecular docking study. Mol Divers. 2020. https://doi.org/10.1007/s11030-020-10038-w

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Theranostics in Metastatic Castrate Resistant Prostate Cancer

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Doi: https://doi.org/10.36255/exonpublications.prostatecancer.theranostics.2021

Abstract: Despite advances in the treatment of localized prostate cancer, many patients progress to metastatic castration-resistant prostate cancer with limited median survival benefits, and significant morbidity. Therefore, efforts to explore new therapeutic modalities for metastatic castration-resistant prostate cancer are urgently needed. A theranostic approach in oncology is based on the principle of imaging a particular molecular target with a diagnostic radioisotope, and then substituting it with a therapeutic isotope to treat a patient who demonstrates sufficient target expression on diagnostic images. Radioisotope pairs are usually chosen in such a way that their physical and chemical properties are similar to ensure the therapeutic agent will be distributed in the same way as the diagnostic agent. This chapter outlines the most recent advances in the use of prostate specific membrane antigen (PSMA) in theranostics with emphasis on ^{177}Lu-PSMA, ^{225}Ac-PSMA and ^{223}Ra-dicloride. The clinical utility of these radioisotopes along with their limitations and future perspectives are discussed.

Keywords: ^{225}Ac-PSMA; ^{177}Lu-PSMA; alpha particle therapy; radioligand therapy; theranostics

In: *Prostate Cancer*. Bott SRJ, Ng KL (Editors). Exon Publications, Brisbane, Australia.
ISBN: 978-0-6450017-5-4; Doi: https://doi.org/10.36255/exonpublications.prostatecancer.2021

INTRODUCTION

Prostate cancer remains an important cause of morbidity and mortality in men (1) with recent global patterns identifying South Africa as one of the countries with the highest mortality (2). The mainstay of localized cancer treatment consists of prostatectomy, active surveillance and/or radiation therapy, whereas metastatic disease is treated with androgen ablation with or without additional agents, such as docetaxel, abiraterone, enzalutamide, cabazitaxel, and sipuleucel-T (Sip-T) (3). Despite the aforementioned therapies, the majority of patients ultimately progress to metastatic castration-resistant prostate cancer with limited median survival benefits and significant morbidity. The median overall survival for metastatic castration-resistant prostate cancer patients ranges from 13–32 months with a 15% 5-year survival rate. Therefore, efforts to explore new therapeutic modalities for metastatic castration-resistant prostate cancer are urgently needed (4).

CONVENTIONAL THERAPIES

Conventional therapy for metastatic prostate cancer consists of androgen deprivation therapy combined with abiraterone acetate plus prednisone, docetaxel, or enzalutamide (4). In the South African setting, initial treatment most commonly includes docetaxel. A recent publication by Abdel-Rahman (5) combined the pooled analysis from three prospective studies which included a total of 1212 patients with metastatic prostate cancer that were treated with the combination of docetaxel and prednisone. Findings indicated a high prevalence of high-grade toxicities, especially neutropenia in older patients. These findings were in line with a number of prior retrospective studies showing a higher risk of treatment toxicities among elderly patients receiving docetaxel for metastatic castration-resistant prostate cancer (5).

THE THERANOSTIC APPROACH

The theranostic approach in oncology is based on the principle of imaging a particular molecular target with a diagnostic radioisotope, and then substituting it with a therapeutic isotope to treat a patient who demonstrates sufficient target expression on the diagnostic images. Radioisotope pairs are usually chosen in such a way that their physical and chemical properties are similar to ensure that the therapeutic agent will be distributed in the same way as the diagnostic agent (Figure 1). Probably the earliest example of this concept is the use of radioactive iodine to image and treat thyroid cancer, where imaging is performed with ^{123}I and therapy with ^{131}I. Another example of such a theranostic pair is ^{68}Ga-PSMA (Gallium-68 prostate specific membrane antigen) and ^{177}Lu-PSMA (Lutetium-177 prostate specific membrane antigen). The radioactive isotope and the target molecule are connected via a ligand or a chelator (6–8).

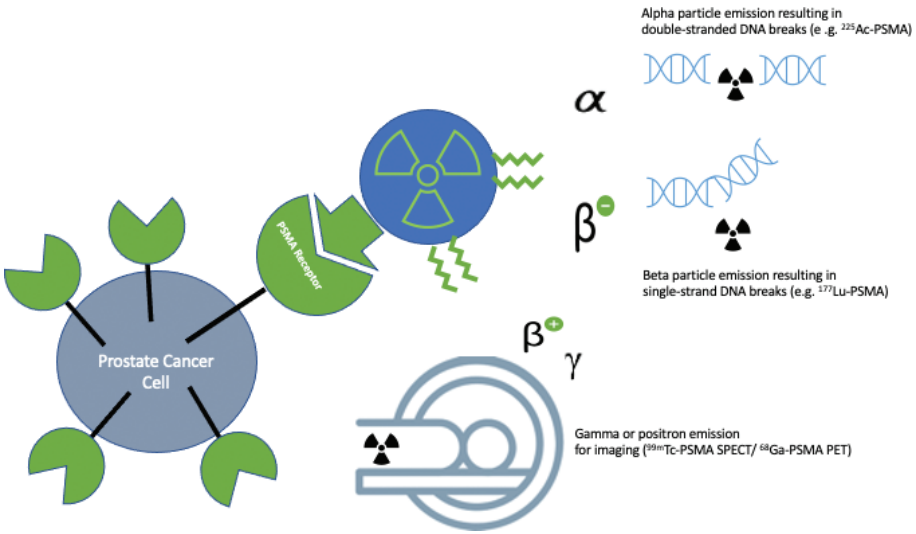

Figure 1. PSMA receptor targeting. Diagrammatic representation of PSMA receptor targeting for cancer detection and / or treatment depending on the radionuclide selection. A theranostic approach consists of first imaging disease presence and extent with a diagnostic isotope prior to targeting disease with a therapeutic isotope.

^{68}GA-PSMA

The superiority of ^{68}Ga-PSMA as an imaging modality in prostate cancer management (for example, bone scintigraphy) has been sufficiently demonstrated in multiple comparative studies as well as in a few systematic reviews and meta-analysis (9–11). A 2020 systematic review and meta-analysis published in European Urology concludes that "Ga-68-PSMA positron emission tomography (PET) improves detection of metastases with biochemical recurrence, particularly at low pre-PET prostate-specific antigen (PSA) levels of >0.2 ng/ml (33%) and 0.2–0.5 ng/ml (45%)". A prospective, multi-center study by Hofman *et al*. (the "proPSMA" study) also convincingly demonstrated the superiority of PSMA-based imaging over computed tomography (CT) and bone scintigraphy (9). Imaging with ^{68}Ga-PSMA has subsequently been included in the guidelines of the European Association of Urology and National Comprehensive Cancer Network (12). When a theranostic approach is planned, any PSMA-based imaging, labelled to Technetium-99 (^{99m}Tc) or Fluorine-18 (^{18}F) may be used for selection and follow-up of patients. However, ^{68}Ga-PSMA has been best validated to date in this setting.

^{18}F-FDG

The use of ^{18}F-FDG (Fluorine-18 Fluorodeoxyglucose) seems valuable in screening patients appropriately for targeted radionuclide therapy, although routine

baseline imaging with both FDG and PSMA may not be practical or cost-effective. Imaging with ^{18}F-FDG PET may have to be reserved for cases with poor clinical and/or biochemical responses despite stable disease, or partial treatment response noted on ^{68}Ga-PSMA. This clinical setting may be indicative of prostate cancer that is no longer expressing PSMA in certain metastatic lesions and incongruent uptake of FDG is generally indicative of a poor prognosis (13–15).

^{177}LU-PSMA

PSMA is a Type II transmembrane glycoprotein, consisting of 750 amino acids that is over-expressed in the vast majority of prostate cancer cells. The PSMA receptor has an internalization process that allows endocytosis of bound proteins on the cell surface into an endosomal compartment, which allows PSMA labelled radioisotopes to be concentrated within the cell. The density of expression of this transmembrane receptor on prostate cancer cells further increases depending on the Gleason score of the prostate cancer, and in castrate- resistant prostate cancers, which makes it an ideal target for radionuclide therapy (16, 17). A small molecule that specifically binds to the PSMA is commercially available as PSMA-11 and PSMA-617. This molecule consists of a glutamate-urea-lysine that has a high affinity and specificity towards PSMA, a chelator (DOTA in case of PSMA-617 for therapy; N,N′-bis [2-hydroxy-5-(carboxyethyl)benzyl] ethylenediamine-N,N′-diacetic acid (HBED-cc) in case of PSMA-11 for diagnosis), and a linker that differs depending on the chelating agent. The linker can be re-designed for optimization of hydrophilicity and biodistribution. PSMA-617 is a theranostic probe that can be used for both imaging and therapy just by changing the radiometal. However, in clinical practice, PSMA-11 is often used for diagnostic staging and the DOTA-analogue PSMA-617 is used for therapy as the biodistribution of the different PSMA ligands is more suitable for each application (18–20).

^{177}Lu is a therapeutic isotope which results in the emission of beta minus particles (β^-) that have a range between 1 and 10 mm with energies between 0.1 and 1 MeV. This results in multiple single strand breaks in the DNA of the targeted cancer cells. A recent review by Jones *et al.* (21) on PSMA theranostics summarized current ongoing trials with ^{177}LuPSMA. In a more recent review, Emmet (22), highlighted the expanding role of PSMA-directed theranostics in prostate cancer as a sensitive diagnostic tool that can be coupled with efficacious and low-toxicity therapeutic options.

Mode of administration of ^{177}Lu-PSMA

Patients are selected based on the expression of the PSMA target after PSMA-based imaging, for example, ^{68}Ga-PSMA PET/CT or ^{99m}Tc-PSMA or ^{18}F-PSMA (Figure 2). Absence of any contra-indications, such as bone marrow suppression or renal impairment, is also evaluated (23, 24). Currently, ^{177}Lu-PSMA radioligand therapy (RLT) is administered on a compassionate basis when all traditional therapies have been exhausted. Its exact sequence in the treatment pathway among well-established therapies is uncertain. The guidelines by the European

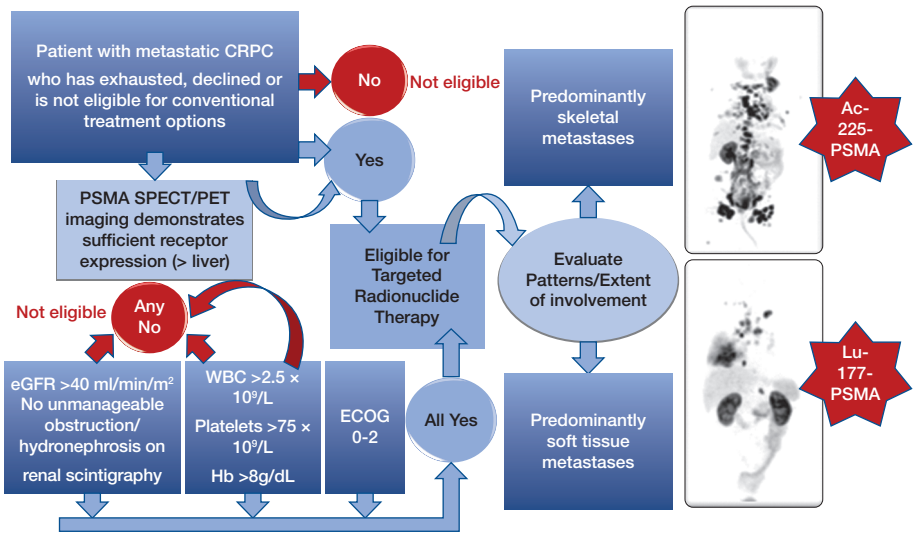

Figure 2. Patient selection for targeted radionuclide therapy. Eligibility evaluation for PSMA radioligand therapy includes demonstrating the presence of sufficient PSMA overexpression on imaging as well as ensuring adequate renal function, bone marrow reserves and ECOG of less than 2. Diffuse skeletal metastases are better suited to treatment with ^{225}Ac-PSMA as opposed to the presence of predominantly soft tissue metastases that is better suited to treatment with ^{177}Lu-PSMA.

Association of Nuclear Medicine (EANM) state that ^{177}Lu-PSMA should be administered in the following way (25): (i) in patients with low cardiovascular risk, 1–2 L normal saline may be given intravenously at 20 cc/min flow rate; and (ii) RLT is administered by slow intravenous injection of ^{177}Lu-PSMA. The following patient-specific recommendations for RLT can be considered:

(i) Diuretics and moderate laxatives can be given after RLT to support clearance of unbound ^{177}Lu-PSMA.
(ii) Cold packs applied to salivary glands could eventually reduce ^{177}Lu-PSMA uptake during the blood pool phase. The value of cold packs is still controversial.
(iii) Prophylactic antiemetic therapy, for example, ondansetron.
(iv) Corticosteroids one day before, and up to several days after RLT are mandatory in case of cerebral, spinal or other metastases with risk of painful or obstructive swelling; otherwise, they are optional and case-dependent.

These guidelines have also been adapted to the South African context and published (26).

Efficacy, safety and outcomes of ^{177}Lu-PSMA

Several retrospective studies performed worldwide have demonstrated promising improvements in progression-free and overall survival (27–30). Hofman *et al*. (24)

performed the first large prospective study at the Peter MacCallum Cancer Centre in Melbourne, Australia. The researchers recruited men with metastatic castration-resistant prostate cancer and progressive disease after standard treatments, including taxane-based chemotherapy and second-generation anti-androgens. Eligible patients had progressive disease as defined by imaging (according to Response Evaluation Criteria In Solid Tumors [RECIST] or bone scan) or new pain in an area of radiographically evident disease, and were required to have an Eastern Cooperative Oncology Group (ECOG) performance status score of 2 or lower. The study participants received up to four cycles of intravenous ^{177}Lu-PSMA-617 with a mean dose of 7.5 GBq/cycle, at six weekly intervals. Primary endpoints consisted of a PSA response according to Prostate Cancer Clinical Trial Working Group criteria (defined as a greater than 50% PSA decline from baseline) and imaging responses (as measured by bone scan, CT, PSMA, and FDG PET/CT) and quality of life (assessed with the EORTC-Q30 and Brief Pain Inventory-Short Form questionnaires). Toxicity was assessed according to Common Terminology Criteria for Adverse Events (CTCAE).

Results indicated that the most common side effects were dry mouth, fatigue and nausea, none of which exceeded grade 2 in severity. In terms of the outcomes, an objective response in nodal or visceral disease was reported in 82% of patients with measurable disease, together with clinically meaningful improvements in pain severity and quality of life, as early as after the second cycle of therapy. The authors concluded that radionuclide treatment with ^{177}Lu-PSMA-617 has a high treatment-response rate with low toxic effects, and reduction of pain in men with metastatic castration-resistant prostate cancer who have progressed after conventional treatments (24).

Emmet *et al*. (31) from the St Vincent's Hospital in Sydney, Australia, conducted a prospective phase 2 study which focused on imaging predictors of treatment response and patterns of disease progression. Eligibility criteria included uptake on PSMA PET above or equal to liver activity, in the absence of any ^{18}F-FDG PET-discordant disease. Study participants received up to 4 cycles of ^{177}Lu-PSMA at 6 weekly intervals, after which imaging was repeated. Treatment response to ^{177}Lu-PSMA was assessed using PSA response and comparison to molecular imaging parameters at baseline. The authors concluded that "PSMA PET plays an important role in predicting treatment response to Lu-PSMA and in identifying subsequent patterns of failure, which may aid in determining the next best treatment options". Several retrospective studies have also suggested similar efficacy and toxicity profiles (31).

A recent meta-analysis and systematic review on RLT with ^{177}LuPSMA for metastatic castrate-resistant prostate cancer suggested that this is an effective treatment of advanced stages of the disease that is refractory to standard therapeutic options and that it has a low toxicity profile (20). In another review on the use of ^{177}LuPSMA in the setting of metastatic prostate cancer, the authors concluded that ^{177}Lu-PSMA RLT is a safe and promising form of treatment especially in patients who have progressed beyond standard treatment. Considering its low toxicity, ^{177}Lu-PSMA RLT is additionally an ideal therapeutic option for patients who do not tolerate docetaxel therapy well or those who have extensive bone marrow involvement. Also, when compared to established systemic therapies, ^{177}Lu-PSMA RLT results in PSA reductions and lengthens overall survival and progression-free survival with low-grade, transient toxicity (32).

^{225}AC-PSMA

The most promising targeted alpha-emitters for RLT included Actinium-225 (^{225}Ac), Bi-213 (^{213}Bi) and Radium-223 (^{223}Ra). Although some initial promising evidence for the use of ^{213}Bi-PSMA was presented (33), it has since been realized that its physical characteristics makes ^{213}Bi a less than ideal candidate. The use of ^{225}Ac-PSMA has been most extensively evaluated in humans and seem to provide the best therapeutic possibilities of the three alpha emitters.

^{225}Ac-PSMA is an alpha-emitter with a relatively long half-life of 9.9 days. Its decay scheme results in the emission of four alpha particles and two beta particles, which together with the long half-life make for a rather cytotoxic radioisotope (34). The therapeutic use of an alpha-emitter offers several advantages. Its high linear energy transfer together with the short radiation range in human tissue results in multiple double-stranded DNA breaks which are largely independent of the cell cycle and oxygenation state, whilst leaving the surrounding unaffected tissues relatively unscathed. It offers an alternative form of therapy when conventional therapies (such as chemotherapy or external radiation) have resulted in resistance. A potential additional advantage is more pronounced abscopal effect when compared to beta radiation in preclinical studies. This effect relates to systemic tumor regression outside of the treated areas and may lead to favorable combinations with immunotherapy, such as immune checkpoint inhibitors (34, 35). Combining alpha-emitters with low molecular weight ligands that are internalized lead to fast tumor uptake and non-target clearance with a more favorable red marrow toxicity profile. Targeted alpha therapies have been in use for decades and have been successfully used in the treatment of brain tumors, neuro-endocrine tumors, prostate cancer and others (34).

Targeted alpha therapy appears to be particularly well-suited for application in combination with other forms of conventional therapy for the ablation of micro-metastases, in patients with diffuse bone marrow infiltration, and in patients who have become resistant to other conventional therapy (34). The practical aspects of ^{225}Ac-PSMA with regards to patient selection, preparation, administration, and minimization of side effects has been described (36) but standardized international guidelines are still lacking.

Dose determination, dosimetry, and administration

Dose determination of ^{225}Ac-PSMA was established by Kratochwil *et al*. (37), based on time-activity curves that made use of dosimetry estimates obtained from serial ^{177}Lu-PSMA scans obtained post-therapy which were then extrapolated to the physical half-life of ^{225}Ac. Fourteen patients were divided into four groups with different empirical doses for salvage therapy as follows: 50kBq/kg (n=4), 100 kBq/kg (n=4), 150kBq/kg (n=2) and 200 kBq/kg (n=4). Treatment response and the presence of any toxicity was retrospectively evaluated. Results indicated the highest radiation dose was received by the salivary glands (2.3Sv), followed by the kidneys (0.7Sv) and the bone marrow (0.05 Sv). The researchers found that xerostomia became the dose-limiting factor at treatment activities above 100 kBq/kg/cycle. Therapy administered at a dose of 100 kBq/kg resulted in a significant decline in s-PSA, which had a duration of less than 4 months, which was then

improved with further cycles administered every two months. Administered activities of 50 kBq/kg resulted in a poor tumor control with no occurrence of side effects. Based on the aforementioned data, 100 kBq/kg/cycle was determined as the optimal dose for human use with intervals of eight weeks in-between doses (37). A recently published 'image of the month' demonstrated the feasibility of image-based dosimetry with ^{225}Ac-PSMA quantitative SPECT (single photon emission computed tomography) (38). Micro dosimetry calculations have also been calculated with Monte Carlo simulations (39).

Clinical evidence with ^{225}Ac-PSMA

Clinical evidence has been limited mostly to retrospective observational studies at this point, with the majority of treated patients having received ^{225}Ac-PSMA in a salvage therapy setting. A paper by Kratochwil and colleagues (40) evaluated tumor control duration and efficacy in a group of 40 patients with metastatic castrate-resistant prostate cancer. A dose of 100 kBq/kg was administered at intervals of two months for three cycles, and six-month follow-up evaluation performed with PSMA-based PET or SPECT imaging. Interim evaluations consisted of s-PSA and full blood count results, done monthly.

Outcomes considered consisted of the duration of the s-PSA response as well as the progression-free response based on the six-month radiological response. Their results demonstrated that 87% of included patients (n=38) demonstrated a decrease in s-PSA and that 63% of patients (n=33) demonstrated a s-PSA response of greater than 50%. Median duration of tumor control was nine months, with five patients even exceeding two-year survival. This is indeed a remarkable response considering the very late stage of disease (when all other treatment options had been exhausted) in this group of patients when compared to conventional therapies used earlier-on in the treatment landscape. Such treatments include abiraterone, demonstrating a median duration of tumor control of 10 months, and docetaxel of 6.5 months. Xerostomia was the main side effect and led to discontinuation of therapy in four patients. The authors commented on a perceived higher efficacy compared to ^{177}Lu-PSMA with salivary gland toxicity as the major limiting factor. The surrogate markers s-PSA and radiological progression-free survival seemed to correspond well to the duration of tumor control (40).

Sathekge *et al*. published a study based on 57 patients, started on doses of either 10MBq (in cases such as those with a high bone tumor burden) or 8MBq which was then de-escalated based on the remaining tumor burden and occurrence of side effects. Radiological measurements and s-PSA were used as surrogate markers and patients were divided into two groups based on previous therapies received. Group A consisted of patients who received combinations of conventional therapy such as surgery, radiation therapy and/or androgen deprivation therapy. A 71% decrease in tumor markers was found in group A, compared to a 92% response rate in Group B that consisted of patients who received minimal or no previous therapy. The majority of these patients reported an improvement in Quality of Life (with decreased bone pain) and minimal side effects. Group B also included patients who were chemotherapy naïve, due to ineligibility or unwillingness to undergo chemotherapy, who demonstrated significantly higher response rates and even complete responses (41).

Another publication on a larger patient cohort by the same group reports on possible outcome predictors in patients with metastatic castrate-resistant prostate cancer treated with ^{225}Ac-PSMA. The study population comprised of 73 patients treated with 210 cycles of ^{225}Ac-PSMA-617. Special investigations that were routinely performed included full blood count, kidney function including glomerular filtration rate, liver function tests, s-PSA and ^{68}Ga-PSMA PET/CT. Eighty-three percent of these patients demonstrated a favorable s-PSA response, with greater than 70% demonstrating a decline in s-PSA exceeding 50%. Indicators of a positive treatment response included the magnitude of the s-PSA decline, whereas patients that were previously treated with ^{177}Lu-PSMA had a poorer prognosis. The results further demonstrated a complete treatment response in 29% of participants and estimated the median progression-free survival and overall survival at 15 months and 18 months, respectively. Xerostomia was reported in the vast majority of patients (85%) but was not severe enough to discontinue therapy. No Grade IV bone marrow toxicity was reported, and renal toxicity occurred only in a small number of patients with baseline renal impairment. From these results, it is clear that ^{225}Ac-PSMA-617 is indeed a viable treatment option for patients who have castration-resistant prostate carcinoma and for whom conventional therapy has failed. Treatment response is durable, and the side effects are tolerable (42).

A more recent prospective study from India (43) evaluated response and outcomes in 28 patients recruited with metastatic castrate-resistant cancer. Participants included a mixture of patients who demonstrated resistance to ^{177}Lu-PSMA (n=15, [54%]) and others who had not been previously treated with ^{177}Lu-PSMA (n=13 [46%]), the majority of whom had extensive skeletal metastases on baseline imaging. This study population also included a significant number of patients (72%) with ECOG 3–4 unlike other study populations. A dose of 100kBq/kg was administered at two-month intervals and responses were evaluated based on s-PSA according to the prostate cancer working group criteria (PCWG3). Other parameters that were evaluated included overall survival, progression-free survival, disease control rate, tumor response according to PERCIST 1 criteria and the occurrence of side effects based on the CTCAE v5 criteria. The authors reported a >50% decline in s-PSA for 39% of patients at the end of follow-up, with a median progression-free survival of 12 months and a median overall survival of 17 months. The authors hypothesized that the slightly inferior results, when compared to other groups, could be attributable to the high percentage of patients with high ECOGs (ECOG 3–4) that were included. Decreases in s-PSA was again identified as a good prognostic indicator and vice versa and side effects experienced were limited to Grade I/II toxicity. Sub-group analysis comparing the outcomes of patients with prior exposure to ^{177}Lu-PSMA compared to those naïve to ^{177}Lu-PSMA demonstrated a greater than 50% decline in 53.8% of patients compared to only 26.6% in the previously exposed group. Similar to previous studies that demonstrated promising disease control rates with ^{225}Ac-PSMA, this study also showed low toxicity (43). There are a number of impressive case studies that demonstrate remarkable treatment responses to ^{225}Ac-PSMA in patients with significant visceral metastases, such as brain, lungs and orbital (44–46). The outcomes of several current large multi-center trials are eagerly anticipated.

COMPARING ^{177}LU-PSMA AND ^{225}AC-PSMA

To the best of the authors' knowledge, no prospective head-to-head comparisons have been performed to establish which form of targeted radionuclide therapy is more effective. Theoretically, ^{225}Ac-PSMA produces more double-stranded DNA breaks within the tumor cells, which should lead to a higher efficacy. It is also better suited to patients with significant skeletal metastases as the shorter tissue penetration should spare the bone marrow to a greater extent than ^{177}Lu-PSMA with its β-particle emission. This unfortunately comes at the cost of significant toxicity to the salivary glands with a non-negligible effect on quality of life. Figure 2 suggests a possible pathway for treatment selection in patients with metastatic castration-resistant prostate cancer. Some centers prefer to treat patients with mainly soft tissue involvement with ^{177}Lu-PSMA and to reserve those with extensive skeletal involvement (with or without soft tissue involvement) for therapy with ^{225}Ac-PSMA. The choice would of course further be dictated by what is available, what is funded, and what the patients' preferences are.

^{223}Ra-dicloride

An overview of targeted alpha therapy in the setting of prostate cancer would hardly be complete without mentioning the use of ^{223}Ra (Radium-223). ^{223}Ra is an alpha particle emitter with a physical half-life of 11.4 days which acts on metastatic bone lesions due to its similarities to calcium in that it complexes with hydroxyapatite at areas of increased bone turnover. The ALSYMPCA trial was a large Phase III international multi-center trial that compared the efficacy of ^{223}Ra dichloride to that of a placebo in the setting of metastatic castrate-resistant prostate cancer. Participants consisted of those with symptomatic and progressive disease with at least two skeletal metastases, but without any visceral metastases and with an ECOG up to 2. Results from this trial consisting of over 900 participants demonstrated an improvement in overall survival of 3.6 months when compared to placebo and was subsequently approved for use by the FDA (47). Side effects were mostly related to bone marrow with resultant anemia, lymphopenia and thrombocytopenia. Long-term effects on the emergence of bone marrow-related cancers are unknown considering the lag effect of nearly 20 years needed and there are a number of other concerns and criticisms around this trial. Importantly, the use of bone scintigraphy in combination with CT in the screening process of participants seems sub-optimal in light of better detection modalities such as ^{68}Ga-PSMA PET (48). The other limitation is that treatment is limited to skeletal metastases only and that any visceral metastases (such as lung, liver and brain with major impact on prognosis) are left untreated. The EANM has recently published a procedure guideline on the use of ^{223}Ra-dicloride in the treatment of skeletal metastases in prostate cancer patients (49).

COMBINATION THERAPIES

Some research groups have adopted a tandem approach making use of both ^{177}Lu-PSMA and ^{225}Ac-PSMA in an attempt to increase efficacy whilst minimizing

possible toxicity. In such approach, researchers from the Saarland University, Saarbrücken, Germany, administered 5 MBq of ^{225}Ac-PSMA in combination with 7 GBq of ^{177}Lu-PSMA-617. Results indicated a good s-PSA response after a single course of low activity ^{225}AcPSMA combined with a full activity of ^{177}Lu-PSMA with reduced salivary gland toxicity (50). Kulkarni *et al*. also used a similar approach making use of 2–7 MBq of ^{225}Ac-PSMA in combination with 3–7.5 GBq of ^{177}Lu-PSMA-617 (51).

To date, there have been no clinical trials evaluating the effects of ^{225}AcPSMA in combination with chemotherapy. Often patients have had chemotherapy at the point when they are referred for targeted alpha therapy and good efficacy is still achieved. Currently, it seems that conventional therapies are complimentary to ^{225}Ac-PSMA and vice versa. The best patient management will take place in the setting of a multi-disciplinary team, where treatment options can be introduced as needs arise. External beam radiation therapy is often needed post targeted alpha therapy in instances where one or two metastatic lesions remain which do not justify systemic therapy.

Targeted alpha therapy provides radiation with high linear energy transfer, whereby even a single alpha particle traversing a DNA strand results in double-stranded DNA breaks with blunt edges that are difficult to repair. Despite this effective mechanism, poor response or resistance is not negligible (despite sufficient PSMA expression) and is likely related to DNA repair pathways. A pilot study by Kratochwil *et al*. investigated 10 patients with poor responses to TAT despite sufficient PSMA expression with CT-guided biopsy and targeted next-generation sequencing. Histology could be obtained in seven lesions, which identified increases in the following mutations in DNA-damage recognition: *ATM, CHEK-2 and TP53*. The authors concluded that the frequency of DNA damage-recognition and signaling-checkpoint genes appeared increased in non-responders. Unfortunately, this study was performed in the absence of a control group, which makes the normal prevalence of these phenomena difficult to assess. This group also commented that the occurrence of gene mutations coding for *BRCA1/2* tended to be rare (52). The implications from these findings are that the combination of TAT with PARP-inhibitors and/or immunotherapies may be beneficial under certain conditions, particularly in poor responders.

Future combinations with DNA damage-repair targeting agents such as poly (ADP-ribose)-polymerase inhibitors (PARPi), such as Olaparib, in patients with germline/ somatic mutations of especially *ATM, BRCA1* and *BRCA2* seems to be a reasonable approach, and this will be evaluated in ongoing prospective trials. Combination with immune checkpoint inhibitors also provide a potentially exciting therapeutic development (53). Figure 3 summarizes the potential mechanisms to optimize PSMA therapy based on a diagram by Kumar *et al*. (53).

TREATMENT RESPONSE EVALUATION CRITERIA

In the majority of published trials on targeted alpha therapy, treatment response evaluation is based on criteria similar to those used by the Prostate Cancer Clinical

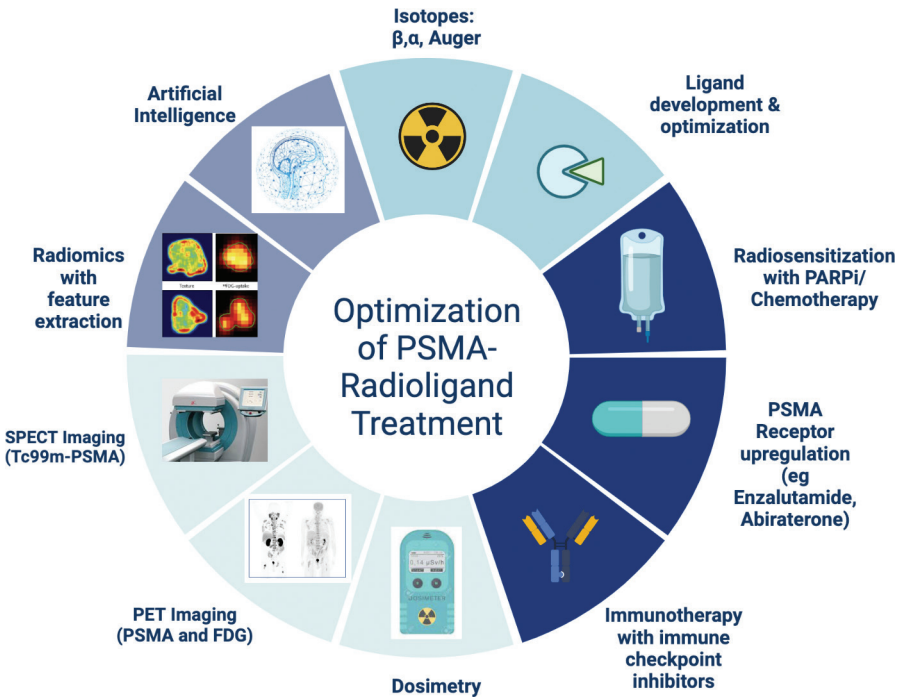

Figure 3. Optimization of prostate cancer treatment with PSMA radionuclide therapy. A combination of various strategies is needed to optimize selection and treatment for patients with metastatic CRPC. TAT combined with chemotherapy, immunotherapy and PARP-inhibitors may increase the effectiveness of therapy over that of a single PSMA-based radionuclide agent, especially in those patients with non-congruent uptake on FDG PET. (*Based on the diagram by Kumar et al Clinical Cancer Research. 2020; 26(12):2774–6.*)

Trials Working Group 2/3 (54) which include clinical and laboratory findings together with conventional imaging modalities (CT, magnetic resonance imaging, and bone scan). Some groups have also included PSMA-based imaging although these have not yet been standardized by means of an international guideline. In a recent editorial published in the Journal of Nuclear Medicine, Fanti *et al.* (55) proposed the development of robust systemic treatment response assessment criteria at the time of PSMA imaging, which they coined "PSMA PET Progression Criteria (PPP)". They propose the following three criteria: (i) the appearance of at least two new lesions that are PSMA-positive and at distant sites; (ii) appearance of one new PSMA-positive lesion, which corresponds to clinical and laboratory data, and which is confirmed either by biopsy or with correlative imaging within three months of the PSMA PET; and (iii) an increase in size or intensity of PSMA uptake in at least one lesion by at least 30% which corresponds to clinical and laboratory data and is confirmed with biopsy or correlative imaging within three months of the PET study (55).

CONCLUSION

It is anticipated that further developments with regards to the type of PSMA and delivery thereof will continue in an effort to maximize the tumor radiation dose whilst limiting radiation to particularly the salivary glands. Improved dosimetry modeling may also assist in the individualization of doses with the need for dose-escalation and de-escalation based on individual patient imaging. This would also apply to establishing ideal treatment intervals and the total number of cycles needed. There is a need for formalized international guidelines with regards to patient selection and treatment regimens as well as the interpretation and reporting of treatment response on PSMA-based imaging. It is further hoped that ^{225}Ac-PSMA will be registered and produced worldwide based on emerging data from prospective randomized trials. It will earn its deserved spot in the treatment landscape of patients with metastatic castrate resistant prostate cancer. It is anticipated that earlier on in the treatment schedule, patients will receive greater benefits either as a single therapy, or in combination with various therapies that may include ^{177}Lu-PSMA, chemotherapy, androgen-deprivation therapy and immune-checkpoint inhibitors.

Conflict of interest: The authors declare no potential conflicts of interest with respect to research, authorship and/or publication of this article.

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REFERENCES

1. Sridhar SS, Freedland SJ, Gleave ME, Higano C, Mulders P, Parker C, et al. Castration-Resistant Prostate Cancer: From New Pathophysiology to New Treatment. Eur Urol. 2014;65(2):289–99. https://doi.org/10.1016/j.eururo.2013.08.008
2. Babb C, Urban M, Kielkowski D, Kellett P. Prostate Cancer in South Africa: Pathology Based National Cancer Registry Data (1986–2006) and Mortality Rates (1997–2009). Prostate Cancer. 2014;1–9. https://doi.org/10.1155/2014/419801
3. Hotte SJ, Saad F. Current management of castrate-resistant prostate cancer. Curr Oncol. 17(Suppl 2): 72–9. https://doi.org/10.3747/co.v17i0.718
4. Gillessen S, Attard G, Beer TM, Beltran H, Bjartell A, Bossi A, et al. Management of Patients with Advanced Prostate Cancer: Report of the Advanced Prostate Cancer Consensus Conference 2019. Eur Urol. 2020;77(4):508–47. https://doi.org/10.1016/j.eururo.2020.01.012
5. Abdel-Rahman O. Efficacy and toxicity outcomes of elderly castrate-resistant prostate cancer patients treated with docetaxel-A pooled analysis of 3 randomized studies. Urol Oncol-Semin Ori. 2020;38(4):210–5. https://doi.org/10.1016/j.urolonc.2019.09.004
6. Yordanova A, Eppard E, Kürpig S, Bundschuh RA, Schönberger S, Gonzalez-Carmona M, et al. Theranostics in nuclear medicine practice. Oncotargets Ther. 2017;10:4821–8. https://doi.org/10.2147/OTT.S140671
7. Drude N, Tienken L, Mottaghy FM. Theranostic and nanotheranostic probes in nuclear medicine. Methods. 2017;130:14–22. https://doi.org/10.1016/j.ymeth.2017.07.004

8. Farolfi A, Lima GM, Oyen W, Fanti S. Molecular Imaging and Theranostics-A Multidisciplinary Approach. Semin Nucl Med. 2019;49(4):247–54. https://doi.org/10.1053/j.semnuclmed.2019.02.002
9. Perera M, Papa N, Roberts M, Williams M, Udovicich C, Vela I, et al. Gallium-68 Prostate-specific Membrane Antigen Positron Emission Tomography in Advanced Prostate Cancer-Updated Diagnostic Utility, Sensitivity, Specificity, and Distribution of Prostate-specific Membrane Antigen-avid Lesions: A Systematic Review and Meta-analysis. Eur Urol. 2019;77(4):403–17. https://doi.org/10.1016/j.eururo.2019.01.049
10. Kimura S, Abufaraj M, Janisch F, Iwata T, Parizi MK, Foerster B, et al. Performance of [68Ga] Ga-PSMA 11 PET for detecting prostate cancer in the lymph nodes before salvage lymph node dissection: a systematic review and meta-analysis. Prostate Cancer Prostatic Dis. 2020;23(1):1–10. https://doi.org/10.1038/s41391-019-0156-z
11. Tan N, Bavadian N, Calais J, Oyoyo U, Kim J, Turkbey IB, et al. Imaging of Prostate Specific Membrane Antigen Targeted Radiotracers for the Detection of Prostate Cancer Biochemical Recurrence after Definitive Therapy: A Systematic Review and Meta-Analysis. J Urol. 2019;202(2):231–40. https://doi.org/10.1097/JU.0000000000000198
12. Mason BR, Eastham JA, Davis BJ, Mynderse LA, Pugh TJ, Lee RJ, et al. Current Status of MRI and PET in the NCCN Guidelines for Prostate Cancer. J Natl Compr Canc Netw. 2019;17(5):506–13. https://doi.org/10.6004/jnccn.2019.7306
13. Jadvar H. Is There Use for FDG-PET in Prostate Cancer? Semin Nucl Med. 2016;46(6):502–6. https://doi.org/10.1053/j.semnuclmed.2016.07.004
14. Wang B, Liu C, Wei Y, Meng J, Zhang Y, Gan H, et al. A Prospective Trial of 68Ga-PSMA and 18F-FDG PET/CT in Nonmetastatic Prostate Cancer Patients with an Early PSA Progression During Castration. Clin Cancer Res. 2020;26(17):4551–8. https://doi.org/10.1158/1078-0432.CCR-20-0587
15. Bauckneht M, Capitanio S, Donegani MI, Zanardi E, Miceli A, Murialdo R, et al. Role of Baseline and Post-Therapy 18F-FDG PET in the Prognostic Stratification of Metastatic Castration-Resistant Prostate Cancer (mCRPC) Patients Treated with Radium-223. Cancers. 2019;12(1):31. https://doi.org/10.3390/cancers12010031
16. Ranjan S. Lutetium-177 prostate-specific membrane antigen-617 theranostics: New therapeutic hope in metastatic castrate-resistant prostate cancer? Indian J Urol. 2020;36(3):227. https://doi.org/10.4103/iju.IJU_193_20
17. Fendler WP, Rahbar K, Herrmann K, Kratochwil C, Eiber M. 177Lu-PSMA radioligand therapy for prostate cancer. Chin J Nucl Medicine Mol Imaging. 2019;39(10):636–40.
18. Zang J, Fan X, Wang H, Liu Q, Wang J, Li H, et al. First-in-human study of 177Lu-EB-PSMA-617 in patients with metastatic castration-resistant prostate cancer. Eur J Nucl Med Mol I. 2019;46(1):148–58. https://doi.org/10.1007/s00259-018-4096-y
19. Das T, Guleria M, Parab A, Kale C, Shah H, Sarma HD, et al. Clinical translation of 177Lu-labeled PSMA-617: Initial experience in prostate cancer patients. Nucl Med Biol. 2016;43(5):296–302. https://doi.org/10.1016/j.nucmedbio.2016.02.002
20. Delker A, Fendler WP, Kratochwil C, Brunegraf A, Gosewisch A, Gildehaus FJ, et al. Dosimetry for 177Lu-DKFZ-PSMA-617: a new radiopharmaceutical for the treatment of metastatic prostate cancer. Eur J Nucl Med Mol I. 2016;43(1):42–51. https://doi.org/10.1007/s00259-015-3174-7
21. Jones W, Griffiths K, Barata PC, Paller CJ. PSMA Theranostics: Review of the Current Status of PSMA-Targeted Imaging and Radioligand Therapy. Cancers. 2020;12(6):1367. https://doi.org/10.3390/cancers12061367
22. Emmett L, Willowson K, Violet J, Shin J, Blanksby A, Lee J. Lutetium 177 PSMA radionuclide therapy for men with prostate cancer: a review of the current literature and discussion of practical aspects of therapy. J Medical Radiat Sci. 2017;64(1):52–60. https://doi.org/10.1002/jmrs.227
23. Hofman MS, Hicks RJ, Maurer T, Eiber M. Prostate-specific Membrane Antigen PET: Clinical Utility in Prostate Cancer, Normal Patterns, Pearls, and Pitfalls. Radiographics. 2017;38(1):200–17. https://doi.org/10.1148/rg.2018170108
24. Hofman MS, Violet J, Hicks RJ, Ferdinandus J, Thang SP, Akhurst T, et al. [177Lu]-PSMA-617 radionuclide treatment in patients with metastatic castration-resistant prostate cancer (LuPSMA trial): a single-centre, single-arm, phase 2 study. Lancet Oncol. 2018;19(6):825–33. https://doi.org/10.1016/S1470-2045(18)30198-0

25. Kratochwil C, Fendler WP, Eiber M, Baum R, Bozkurt MF, Czernin J, et al. EANM procedure guidelines for radionuclide therapy with 177Lu-labelled PSMA-ligands (177Lu-PSMA-RLT). Eur J Nucl Med Mol I. 2019;46(12):2536–44. https://doi.org/10.1007/s00259-019-04485-3
26. Vorster M, Warwick J, Lawal I, Toit P du, Vangu M, Nyakale N, et al. South African guidelines for receptor radioligand therapy (RLT) with Lu-177-PSMA in prostate cancer. S Afr J Surg. 2020;57(4):45–51. https://doi.org/10.17159/2078-5151/2019/v57n4a3107
27. Yadav MP, Ballal S, Tripathi M, Damle NA, Sahoo RK, Seth A, et al.. 177 Lu-DKFZ-PSMA-617 therapy in metastatic castration resistant prostate cancer: safety, efficacy, and quality of life assessment. Eur J Nucl Med Mol Imaging. 2017 Jan;44(1):81–91. https://doi.org/10.1007/s00259-016-3481-7
28. Baum RP, Kulkarni HR, Schuchardt C, Singh A, Wirtz M, Wiessalla S, et al. 177Lu-Labeled Prostate-Specific Membrane Antigen Radioligand Therapy of Metastatic Castration-Resistant Prostate Cancer: Safety and Efficacy. J Nucl Med. 2016;57(7):1006–13. https://doi.org/10.2967/jnumed.115.168443
29. Bräuer A, Grubert LS, Roll W, Schrader AJ, Schäfers M, Bögemann M, et al. 177Lu-PSMA-617 radioligand therapy and outcome in patients with metastasized castration-resistant prostate cancer. Eur J Nucl Med Mol I. 2017;44(10):1663–70. https://doi.org/10.1007/s00259-017-3751-z
30. Gadot M, Davidson T, Aharon M, Atenafu EG, Malki A, Levartovsky M, et al. Clinical Variables Associated with PSA Response to Lutetium-177-PSMA ([177Lu]-PSMA-617) Radionuclide Treatment in Men with Metastatic Castration-Resistant Prostate Cancer. Cancers. 2020;12(5):1078. https://doi.org/10.3390/cancers12051078
31. Emmett L, Crumbaker M, Ho B, Willowson K, Eu P, Ratnayake L, et al. Results of a Prospective Phase 2 Pilot Trial of 177Lu-PSMA-617 Therapy for Metastatic Castration-Resistant Prostate Cancer Including Imaging Predictors of Treatment Response and Patterns of Progression. Clin Genitourin Canc. 2019;17(1):15–22. https://doi.org/10.1016/j.clgc.2018.09.014
32. Kim YJ, Kim Y. Therapeutic Responses and Survival Effects of 177Lu-PSMA-617 Radioligand Therapy in Metastatic Castrate-Resistant Prostate Cancer. Clin Nucl Med. 2018;43(10):728–34. https://doi.org/10.1097/RLU.0000000000002210
33. Sathekge M, Knoesen O, Meckel M, Modiselle M, Vorster M, Marx S. 213Bi-PSMA-617 targeted alpha-radionuclide therapy in metastatic castration-resistant prostate cancer. Eur J Nucl Med Mol. 2017;44(6):1099–100. https://doi.org/10.1007/s00259-017-3657-9
34. Morgenstern A, Apostolidis C, Kratochwil C, Sathekge M, Krolicki L, Bruchertseifer F. An Overview of Targeted Alpha Therapy with 225 Actinium and 213 Bismuth. Curr Radiopharm. 2018;11(3):200–8. https://doi.org/10.2174/1874471011666180502104524
35. McDevitt MR, Ma D, Lai LT, Simon J, Borchardt P, Frank RK, et al. Tumor Therapy with Targeted Atomic Nanogenerators. Science. 2001;294(5546):1537–40. https://doi.org/10.1126/science.1064126
36. Ahmadzadehfar H, Rahbar K, Essler M, Biersack HJ. PSMA-Based Theranostics: A Step-by-Step Practical Approach to Diagnosis and Therapy for mCRPC Patients. Semin Nucl Med. 2019;50(1):98–109. https://doi.org/10.1053/j.semnuclmed.2019.07.003
37. Kratochwil C, Bruchertseifer F, Rathke H, Bronzel M, Apostolidis C, Weichert W, et al. Targeted α-Therapy of Metastatic Castration-Resistant Prostate Cancer with 225 Ac-PSMA-617: Dosimetry Estimate and Empiric Dose Finding. J Nucl Med. 2017;58(10):1624–31. https://doi.org/10.2967/jnumed.117.191395
38. Gosewisch A, Schleske M, Gildehaus FJ, Berg I, Kaiser L, Brosch J, et al. Image-based dosimetry for 225Ac-PSMA-I&T therapy using quantitative SPECT. Eur J Nucl Med Mol. 2020;1–2. https://doi.org/10.1007/s00259-020-05024-1
39. Gabiña PM, Roeske JC, Mínguez R, Rodeño E, Iturriaga AG de. Microdosimetry-based determination of tumour control probability curves for treatments with 225 Ac-PSMA of metastatic castration resistant prostate cancer. Phys Med Biol. 2020;65(23):235012. https://doi.org/10.1088/1361-6560/abbc81
40. Kratochwil C, Bruchertseifer F, Rathke H, Hohenfellner M, Giesel FL, Haberkorn U, et al. Targeted α-Therapy of Metastatic Castration-Resistant Prostate Cancer with 225Ac-PSMA-617: Swimmer-Plot Analysis Suggests Efficacy Regarding Duration of Tumor Control. J Nucl Med. 2018;59(5):795–802. https://doi.org/10.2967/jnumed.117.203539

41. Sathekge M, Bruchertseifer F, Knoesen O, Reyneke F, Lawal I, Lengana T, et al. 225Ac-PSMA-617 in chemotherapy-naive patients with advanced prostate cancer: a pilot study. Eur J Nucl Med Mol I. 2019;46(1):129–38. https://doi.org/10.1007/s00259-018-4167-0
42. Sathekge M, Bruchertseifer F, Vorster M, Lawal I, Knoesen O, Mahapane J, et al. Predictors of overall and disease free survival in metastatic castration-resistant prostate cancer patients receiving 225Ac-PSMA-617 radioligand therapy. J Nucl Med. 2019;61(1):jnumed.119.229229. https://doi.org/10.2967/jnumed.119.229229
43. Yadav MP, Ballal S, Sahoo RK, Tripathi M, Seth A, Bal C. Efficacy and safety of 225Ac-PSMA-617 targeted alpha therapy in metastatic castration-resistant Prostate Cancer patients. Theranostics. 2020;10(20):9364–77. https://doi.org/10.7150/thno.48107
44. Sathekge MM, Bruchertseifer F, Lawal IO, Vorster M, Knoesen O, Lengana T, et al. Treatment of brain metastases of castration-resistant prostate cancer with 225Ac-PSMA-617. Eur J Nucl Med Mol I. 2019;46(8):1756–7. https://doi.org/10.1007/s00259-019-04354-z
45. Maserumule LC, Mokoala KMG, Hlongwa KN, Ndlovu H, Reed JD, Ismail A, et al. Exceptional initial response of prostate cancer lung metastases to 225Ac-PSMA: A case report. CPCCR. 2021;3:100038. https://doi.org/10.1016/j.cpccr.2020.100038
46. Parihar AS, Chandekar KR, Singh H, Sood A, Mittal BR. Orbital and brain metastases on 68Ga-PSMA PET/CT in a patient with prostate carcinoma refractory to 177Lu-PSMA and 225Ac-PSMA therapy. Asia Ocean J Nucl Med Biol. 2021;9(1):67–70.
47. Heidegger I, Pichler R, Heidenreich A, Horninger W, Pircher A. Radium-223 for metastatic castration-resistant prostate cancer: results and remaining open issues after the ALSYMPCA trial. Transl Androl Urol. 2018;7(Suppl 1):S132–4. https://doi.org/10.21037/tau.2017.10.06
48. Sartor O, Coleman RE, Nilsson S, Heinrich D, Helle SI, O'Sullivan JM, et al. An exploratory analysis of alkaline phosphatase, lactate dehydrogenase, and prostate-specific antigen dynamics in the phase 3 ALSYMPCA trial with radium-223. Ann Oncol. 2017;28(5):1090–7. https://doi.org/10.1093/annonc/mdx044
49. Poeppel TD, Handkiewicz-Junak D, Andreeff M, Becherer A, Bockisch A, Fricke E, et al. EANM guideline for radionuclide therapy with radium-223 of metastatic castration-resistant prostate cancer. Eur J Nucl Med Mol Imaging. 2018;45(5):824–45. https://doi.org/10.1007/s00259-017-3900-4
50. Khreish F, Ebert N, Ries M, Maus S, Rosar F, Bohnenberger H, et al. 225Ac-PSMA-617/177Lu-PSMA-617 tandem therapy of metastatic castration-resistant prostate cancer: pilot experience. Eur J Nucl Med Mol Imaging. 2020;47(3):721–8. https://doi.org/10.1007/s00259-019-04612-0
51. Kulkarni HR, Singh A, Schuchardt C, Niepsch K, Sayeg M, Leshch Y, et al. PSMA-Based Radioligand Therapy for Metastatic Castration-Resistant Prostate Cancer: The Bad Berka Experience Since 2013. J Nucl Med. 2016;57(Supplement_3):97S-104S. https://doi.org/10.2967/jnumed.115.170167
52. Kratochwil C, Giesel FL, Heussel C-P, Kazdal D, Endris V, Nientiedt C, et al. Patients Resistant Against PSMA-Targeting α-Radiation Therapy Often Harbor Mutations in DNA Damage-Repair-Associated Genes. J Nucl Med. 2020;61(5):683–8. https://doi.org/10.2967/jnumed.119.234559
53. Kumar ASR, Hofman MS. Mechanistic Insights for Optimizing PSMA Radioligand Therapy. Clin Cancer Res. 2020;26(12):2774–6. https://doi.org/10.1158/1078-0432.CCR-20-0209
54. Scher HI, Morris MJ, Stadler WM, Higano C, Basch E, Fizazi K, et al. Trial Design and Objectives for Castration-Resistant Prostate Cancer: Updated Recommendations From the Prostate Cancer Clinical Trials Working Group 3. J Clin Oncol. 2016;34(12):1402–18. https://doi.org/10.1200/JCO.2015.64.2702
55. Fanti S, Hadaschik B, Herrmann K. Proposal of Systemic Therapy Response Assessment Criteria in time of PSMA PET/CT imaging: PSMA PET Progression (PPP). J Nucl Med. 2019;61(5):678–82. https://doi.org/10.2967/jnumed.119.233817

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Adenocarcinoma of the Prostate: Future Directions for Translational Science

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Doi: https://doi.org/10.36255/exonpublications.prostatecancer.translationalscience.2021

Abstract: Adenocarcinoma of the prostate is a common malignancy affecting one in nine men, with six of every 10 cases identified in men older than 66 years, and more adversely affects African American males. It remains less common in men under the age of 40. The age adjusted incidence is increasing with the application of prostate specific antigen (PSA) as a biomarker. PSA helps identifying the disease at an early stage, which is treatable and curable with traditional therapies. However, a significant percentage of men present with high Gleason grade and advanced disease, with lower PSA, and younger age at presentation. These patients can have a compromised outcome. Once again, we are evaluating patients under the age of 50 with advanced disease due in part to inconsistent application of clinical screening. More effort is needed for high-risk patients to

In: *Prostate Cancer*. Bott SRJ, Ng KL (Editors). Exon Publications, Brisbane, Australia.
ISBN: 978-0-6450017-5-4; Doi: https://doi.org/10.36255/exonpublications.prostatecancer.2021

provide timely, meaningful intervention and effective therapy. In this chapter, we review the status of therapy for standard and high-risk patients, and strategies for translational science for patients at risk of compromised outcome and treatment failure.

Keywords: biomarkers; castration-resistant prostate cancer; hormone-sensitive disease; neuroendocrine expression; therapeutic resistance

INTRODUCTION

Adenocarcinoma of the prostate affects one in nine men and adversely affects African Americans (1). Local therapy including surgery and modern radiation therapy are curative, with success in low and favorable intermediate risk patients, which include more than 50% of patients who acquire the disease. This has driven a conversation of therapeutic nihilism for favorable patients. While clinical physicians have always weighed risks and benefits of therapy in juxtaposition with medical comorbidities, arguments that the impact on normal tissue by therapy outweigh the benefits of identification of the disease have become a more visible narrative promoting therapeutic nihilism and a decrease in patient screening. This has led to an increase in the identification of high-risk patients who may have benefited from earlier and more timely intervention before the disease became more advanced and less treatable/curable (2–5). Clinical colleagues continue to refine therapy techniques to decrease therapeutic morbidity including image validation of surgical margins and compressed courses of image-guided radiation therapy. In addition, improvements in systemic therapy need to be developed and applied in a timely strategic manner to improve the care of those at most extreme risk of therapy failure despite traditional therapy. These include strategies for incorporation of validated genomic and proteomic biomarkers into clinical decision care paths (6).

CURRENT PATIENT MANAGEMENT

Groups of investigators vary in the interpretation and definition of low, intermediate, and high-risk disease. For the patient at diagnosis, most study groups and national organizations consider high risk disease to be patients with PSA > 20 at presentation with Gleason score of 8–10 and clinical stage >= T3 (7). The National Comprehensive Cancer Network (NCCN) separate high risk from very high risk by T stage with T3A being high risk and T3B-T4 being very high risk (7). Other groups including the American Urological Association, the European Association of Urology, and the NRG cooperative group of the National Clinical Trials Network (NCTN) have subtle variation in criteria, nevertheless patients with more locally advanced disease, positive lymph nodes, and significant elevation in PSA with high Gleason grade are considered high risk patients (8–10).

Hormone-sensitive disease

These patients are identified at the time of initial diagnosis or early in their disease course at the time of treatment failure. The disease almost uniformly remains responsive to hormonal therapy and nearly all forms of therapy either for local disease or oligometastatic disease as part of primary management. The application of hormone therapy is highly effective in both primary and metastatic disease (11). The challenge for patient outcome is the sequelae of hormone management applied for a protracted time period. Because of the influence of surgery/orchiectomy, the clinical application of testosterone interruption therapy was thought optimally delivered as permanent therapy (12,13). With the advent of testosterone antagonist therapy, therapy did not have to be applied on a permanent basis and hormone treatment could be titrated.

Hormone therapy

Investigators were initially influenced by analogies drawn to breast cancer treatment. In breast cancer, hormone therapies are applied for years with evidence that longer duration is superior to shorter duration. Although the sequelae and limited tolerance of therapy are acknowledged, breast cancer care continues to apply hormone therapy for extended periods of time with half and full decades of treatment. The sequelae of hormone therapy in men treated for extended period for prostate cancer are visible and leave fingerprints not easily removed. There are cardiovascular, neurocognitive, and muscular-skeletal risks associated with protracted hormone therapy prompting investigators to re-visit the application of hormone therapy and to titrate the duration of hormone therapy in selected clinical situations when possible. The interruption of testosterone signaling pathways appears to have a negative impact on coronary artery health and may have a direct or indirect effect secondary to cardiovascular health on the central nervous system (14). Hormone therapy can demineralize bones and decrease muscle mass further compromising cardiovascular health (15). Hormonal therapy has both direct and indirect impact on carbohydrate metabolism resulting in increased adipose deposition, fluid retention, and weight gain creating additional challenges in maintaining both cardiovascular and muscular-skeletal health (16). The mechanism of this effect is multi-focal, however decrease in muscle mass limits storage capacity for glycogen thus promoting adipose deposition through gluconeogenesis-associated pathways. Investigators continue to evaluate the potential benefits of hormone therapy coupled with therapeutic titration (how much is enough) and different strategies.

Alternative strategies

Adequate alternatives to hormonal therapy remain an objective for translational science. The addition of hormone therapy to radiation therapy for unfavorable intermediate risk and high-risk patients has led to a survival benefit for these patients (17–18). The benefit is always balanced with therapy sequelae. Strategies for achieving the benefits without sequelae are goals for translational science

moving forward for this patient population given the success of therapy. Our assumption has been that the mechanism of action for therapy is directly associated with testosterone interruption. This is an accepted validated mechanism; however, it is time to revisit this paradigm as alternative and additive thought processes may prove to be of additional importance. Hormone ablation therapy may function through multiple mechanisms including interruption of molecular cell adhesion pathways which play an important role in the development of tumor angiogenesis. In laboratory experiments, hormone ablation therapy appears to alter cell adhesion, preventing tumor growth and development. Although therapies directed at angiogenesis hold promise (19–21), the impact traditionally placed emphasis on altering the established tumor microvascular compartment. Strategies for cell adhesion modification placed emphasis on limiting tumor cell growth prior to the development of tumor angiogenesis. This may explain in part why cell adhesion modification with Casodex (bicalutamide) is effective because it limits tumor cell development before tumor vessels can mature (22–23). This is a possible additional benefit for radiotherapy in addition to testosterone interruption. Therefore, adhesion molecules such as integrins may be important along several steps of the angiogenesis pathway, however the most important step may be at the initiation of tumor cell adhesion. Coupled with radiation therapy, there was significant increase in tumor cell kill including *in vivo* tumor explants of prostate cancer cells with cell adhesion based therapeutic modulation (24–26). Therefore, strategies to apply therapy to specific tumor cell adhesion molecules, including integrin modulation, appear potentially effective in mimicking some of the impact of hormone ablation therapy but hormone signaling does many more things than simply regulate cell adhesion. Initial clinical trials using COX-2 inhibition in multiple disease areas were less successful (27–28), however, coupling cell type specific therapy with radiation treatment may help titrate hormone therapy and provide alternatives to management moving forward. If adequate alternative therapy, such as cell adhesion modulation as a co-partner with radiation therapy, can be identified, hormonal ablation therapy can be deferred to second line management as disease would be hormone-sensitive and vulnerable to therapy. This would position the therapy community quite well and delay the onset of castration-resistant disease secondary to chronic application of hormone treatment. Alternative strategies including the use of novel androgen receptor signaling inhibition with agents such as abiraterone acetate and enzalutamide may also hold promise in early phase hormone sensitive disease as an alternative to Lupron and other current hormone application strategies (29, 30).

Radiation therapy

Improvements in radiation therapy technology have provided significant advances in patient care. Therapy can now be applied in high doses in a safe manner through intensity modulation and daily image guidance which is both nimble and accurate. Using volume modulated arc therapy, concerns of intra-fractional motion of targets are eased due to the speed of treatment with therapy now delivered in a few minutes once targets have been confirmed with image guidance (31).

Investigators are now delivering higher daily treatment dose to targets due in large part to the confidence in daily treatment reproducibility and accuracy in normal tissue avoidance (32–33). In the past, hormone therapy was thought to be an excellent adjunct to care, perhaps in part to limitations in therapy technology. Coupled with modern imaging and improvements in image tracers for PET including therapeutic application of radio pharmacy, radiation therapy may evolve into the initial sole modality of care with hormone and hormone-associated analog therapy moved to second line management, even for hormone-sensitive disease (5).

Hormone-resistance disease

Although nearly all patients respond to hormone androgen deprivation therapy, over time patients with metastatic disease and protracted hormone therapy ultimately develop castration-resistant disease refractory to more traditional therapies. Although prostate cancers historically metastasize to bone as a preferred site of disease, clinicians are now seeing soft tissue disease including end organ parenchyma and the central nervous system (34–37). This may be in part driven by alteration of the bone marrow environment by protracted hormonal application thus generating alteration in soft tissue parenchymal microenvironment promoting tumor growth. This may also be due to the propensity of tumors that undergo treatment-induced neuroendocrine differentiation to metastasize to visceral organs such including the liver (37). The development of this situation is accompanied in parallel by an accumulation of various gene mutations, chromosomal translocations, and increased aberrant DNA repair mechanisms and lineage plasticity within the tumor population that permit the hormone insensitive cells to thrive and proliferate (38–39). These tumors have a predilection to express homologous recombination genes independent of processes associated with repair, therefore more vulnerable to mutations and malevolent clinical behavior. This population of patients continue to have guarded outcome, therefore a real opportunity for improvement in clinical care. In addition, patients with alterations in DNA repair pathways may respond better to immunotherapies due to a higher mutational load. Application of androgen receptor directed therapies in the early phase of castrate resistance can generate a response to therapy, however, the response is not uniformly durable, therefore alternate strategies need to be developed (29).

Tumor metabolism

Tumor metabolism pathways are also altered in advanced prostate cancer. Pathways supporting glycolysis appear promoted in prostate tumor cells. Among potentially important pathways includes fructose-biphosphate aldose A (ALDOA). Aldolase A is encoded by ALDOA and is an important enzyme on the glycolysis pathway. Recent evidence suggests that ALDOA is an oncogene and upregulation of it is associated with prostate cancer growth, metastasis, and poor survival. This raises the potential of a metabolic target for prostate cancer care moving forward (40).

Androgen and androgen receptors

Androgen signaling drives prostate cancer development and disease progression and is thought to be responsible for inducing pathways towards castration resistant disease. The androgen receptor functions as a transcription factor and activates downstream signaling associated with disease progression which is interrupted by androgen deprivation therapy. Endogenous androgens include testosterone and dihydrotestosterone. Even in their absence, resistance can develop, and hormone therapy becomes ineffective even though the androgen signaling pathways remain active (41). Alterations in the androgen receptor have been identified which are potentially responsible or work in parallel to the development of castrate resistant disease. An initial example of alteration was the identification of additional X chromosome copy numbers in the AR gene locus in patients with castrate resistant disease (42, 43). Translational science has identified AR phosphorylation sites/kinase function which are thought to maintain transcriptional activity in this setting including the generation of altered binding sites (44). It is thought that hypoxia inducible factor (HIF) functions through this mechanism. In the absence of oxygen, HIF is activated, translocated to the nucleus, and activates genes associated with invasion and tumor cell survival (45). Expression of HIF is associated with poor prognosis in patients with prostate cancer as ADT in hypoxia is thought to induce adaptive androgen/AR therapeutic independence, thus invite resistance to therapy (46).

Activated kinase

Activated kinase activity is also thought to play an important role in castrate-resistant disease (47). Therefore, modern transcriptomic profiling can be applied to tissue of patients with known castrate-resistant disease to see if repetitive common pathways can be identified to define actionable targets for application of therapy. Investigators have identified in tissue samples of patients with castrate-resistant prostate cancer amplification of extracellular signal-regulated kinase 1 (*ERK*1) in a large cohort of patients (48, 49). Elevated levels of phosphorylated ERK1 and ERK2 have been found in tissue samples of patients who were with high-risk features post prostatectomy and outcome were aligned with ERK expression more than clinicopathological features (50). Therefore, identifying both appropriate patients and targets that align with expression products are needed to improve outcomes. Approved therapies such as Trametinib may be applicable in this setting. In our laboratory, we have identified subsets of cell lines resistant to radiation therapy which can be made more sensitive to treatment with down regulation of ERK 1 and 2 (51).

Neuroendocrine and resistance to therapeutics

Neuroendocrine features are now being identified in prostate cancer and they impose a guarded clinical outcome (52–54). The neuroendocrine cells are found to increase in prostate adenocarcinoma (55–57). The expansion of NE cells in prostate cancer may be derived from preexisting NE cells in the normal or

neoplastic prostate cells (58, 59), or from treatment-induced differentiation (60–64). Neuroendocrine progenitor cells are a limited percentage of epithelial cells and reside in all anatomical zones of the prostate gland. They have limited proliferative potential and are devoid of the androgen receptor with limited to no capacity to generate PSA (55). The neuroendocrine cells contain neurosecretory granules and express neural peptide hormones including bombesin/gastrin secreting releasing peptide (GRP), neurotensin (NT), serotonin, calcitonin, and parathyroid hormone related peptide (PTHrP) (65). These cells express survival genes including Bcl-2. In our laboratory, we have identified modified cell lines that demonstrate resistance to radiation therapy. These cells interestingly demonstrate the ability to undergo epithelial-mesenchymal transition. These cells can be made sensitive to treatment with down regulation of Bcl-2 (65, 66).

Poly (ADP-ribose) polymerase

It is important to develop pathways of therapy that are independent of hormone-related signaling strategies. A potential area of improvement is the use of poly (ADP-ribose) polymerase (PARP) inhibitors. These compounds impact DNA base excision repair, transcription, replication, genomic stability, and cell death (67, 68). Because of their impact generated in the background of fundamental basic science, PARP inhibition holds promise in multiple disease sites including prostate cancer (69–71). One of the involved mechanisms is the mammalian target of rapamycin pathway (mTOR) (72). While PARP-1 activation may promote tumor cell growth, inhibition of PARP inhibits tumor growth and promotes cell death particularly when defect of other genes such as *BRCA1* and *BRCA2* or *ATM* (Ataxia–telangiectasia mutated) exist (73, 74). Inhibition of PARP in malignancy with *BRCA1* and *BRCA2* mutations shows convergence of AR signaling pathways and DNA damage response. Down-regulating homologous recombination genes in castrate-resistant tumor with AR-directed therapy may serve to further sensitize tumor to PARP inhibition as a two-step process including both therapies (75). Early clinical trials are favorable in selected patients who have homozygous deletions, deleterious mutations, or both, in *BCRA* 1/2, *ATM* (Fanconi) and *CHEK2*, identified using next generation sequencing in castrate-resistant patients including those who had received previous therapy including docetaxel (76, 77). The next generation of PARP inhibitors are demonstrating response is previously treated patients (78). In our laboratory, we have identified cell lines resistant to radiation therapy that can be made more sensitive to treatment with the addition of PARP inhibitors, therefore a potentially promising addition to the care of high-risk patients.

Immunotherapy

Cellular immunotherapy with sipuleucel-T has been applied to patients with metastatic castrate resistant prostate cancer (79). Though the first ever FDA-approved immunotherapy, Sipuleucel-T, was approved in the setting of castration resistance prostate cancer (albeit only mediating a moderate delay in mortality) (80), other now conventional cell-based (for example, chimeric antigen receptor [CAR]-T

cells) and checkpoint blockade immunotherapies (for example, PD-1/PD-L1, CTLA-4 antibodies) that have revolutionized the treatment of other malignancies have yet to be effective or approved in the setting of prostate cancer (81, 82). This is possibly a consequence of the immunologically "cold" landscape of prostate cancer that is devoid of cytotoxic T lymphocytes and infiltrated with immunosuppressive myeloid cell populations. Combining immunotherapeutic agents with other approved therapies for castration-resistance prostate cancer, however, appears to be a promising strategy and is currently under investigation (83). Patients with alterations in DNA repair genes may respond better to immunotherapies due to a higher tumor mutational burden and combining immune checkpoint blockade with PARP inhibitors may further improve the treatment outlook of this subset of castration resistance prostate cancer patients. Release of tumor antigens and immunostimulatory molecules following radiation-induced cell death may also boost the impact of immunotherapy regimens by increasing tumor immunogenicity (84–86). In addition, therapies that indirectly (for example, chemotherapies) or directly (for example, CSF1-R, CCL2 antagonists) target suppressive myeloid populations could further enhance T cell function and as a result immunotherapy efficacy.

Imaging and theranostics

Improvements in imaging are playing an important role in patient management for prostate cancer. Magnetic resonance imaging is fused into ultrasound to provide accuracy in biopsy, identify aggregates of disease including extraprostatic extension of disease, involvement of the neurovascular bundle, and potentially identify areas of high-risk disease less obvious in a background of low-risk disease (86). This has considerable influence in the planning of radiation therapy and moves patients with higher risk to radiation therapy as opposed to surgery for primary management. In addition, this has served to improve the accuracy of radiation therapy including brachytherapy both in planning and daily execution of therapy. Metabolic imaging with Axumin (fluciclovine PET/CT scan) (87) and PSMA PET/CT scan (88) is teaching us more accurate location of lymph node anatomy further improving the accuracy of radiation therapy. Further improvements in imaging with respect to identification of disease and response to therapy will be discussed in the section on hormone resistant disease.

The Radiographic Assessments for detection of Advanced Recurrence (RADAR) Groups 1 and 2 have identified fundamental elements of imaging of prostate cancer including technetium bone scan, magnetic resonance imaging, and computer tomography (89, 90). In the group 2 review, important aspects of next generation imaging (NGI) with novel PET radioligands were identified as being important next steps for both imaging disease and possibly being used as vehicles for therapy as these strategies mature for the application of radiopharmacy (89). Imaging with bone scintigraphy with technetium-labeled poly phosphates and diphosphates (Tc 99/Tc MDP) is a well-established imaging strategy for both identification of bone metastasis and evaluating response and/or disease progression. Alpha emitting Radium 223 has been used to treat

bone metastasis. The breakdown product of Radium is strontium which is deposited in bone and subsequently affects bone metastasis as a secondary indirect event (91).

Prostate specific membrane antigen (PSMA) has emerged as an important biomarker for prostate cancer management and may serve an important role for both imaging and the emerging field of theranostics combining imaging and infusional radiation therapy (92). Technetium is a bone-seeking isotope active in areas of new bone formation, therefore identifying areas of tumor in an indirect manner as disease promotes new bone formation as a response to injury (93, 94). In adults, new bone formation can be associated with degenerative bony changes or metastatic disease, however in context the test is helpful in evaluating potential areas of metastatic disease. F-Na-18 fluoride can be applied with PET to identify bone metastasis similar to technetium and may also provide a quantitative basis for measuring the volume of disease in bone because of three-dimensional volumetrics (95). The challenge for these compounds is they only measure disease in bone. Given the changes in pattern of care moving forward including failure and disease progression in soft tissue, imaging vehicles of the future will require strategies for imaging all sites of disease for quantification of the volume of disease and response assessment. F18-fluciclovine (F18-FACBC) images protein synthesis and can be applied with PET CT and PET MR (96). This can image prostate recurrence without significant uptake in the bladder and has the potential of providing quantitative metrics for the volume of disease. F18/C11-Choline and C13-acetate both target phospholipid synthesis (97). Both can identify recurrence of disease and provide quantitative metrics. Bladder accumulation and short half-life of C13 acetate can limit utility of clinical application. PSMA-targeting agents have been developed with imaging using SPECT-CT and PET-CT which can be applied for imaging of recurrence and possibly therapy (98, 99). Serial imaging with SPECT may be used moving forward with image fusion to perform quantitative dosimetry. Dose to target volume as well as dose migration to unintended sites can calculate dose to target in a manner similar to teletherapy and brachytherapy and use radiosurgery to augment dose to target as needed for treatment of oligometastasis. This approach would be used by replacing the radionucleotide Ga 68 with either a beta emitting nuclide such as lutetium 177 (Lui 177) or an alpha emitter such as actinium 225 (Ac 225 or bismuth 213 (Bi 213) (100). Alpha emitters may have a theoretical advantage as tumoricidal effects may be independent of both cell cycle and the oxygen effect (100). Early trials with AC 225 have demonstrated response in patients with castrate resistant prostate disease with xerostomia as the primary point of toxicity due to accumulation of isotope in salivary tissue. The alpha particle therapy appears more effective in chemotherapy naïve disease. Therefore, the future may include the application of tools for both imaging and therapy through similar processes with imaging used to both identify disease and assess dose to volume using stereotactic therapy to augment dose to areas of limited uptake. Through this prism the radiation oncology/radiology partnership may provide the upfront “chemotherapy” (systemic therapy) of the future.

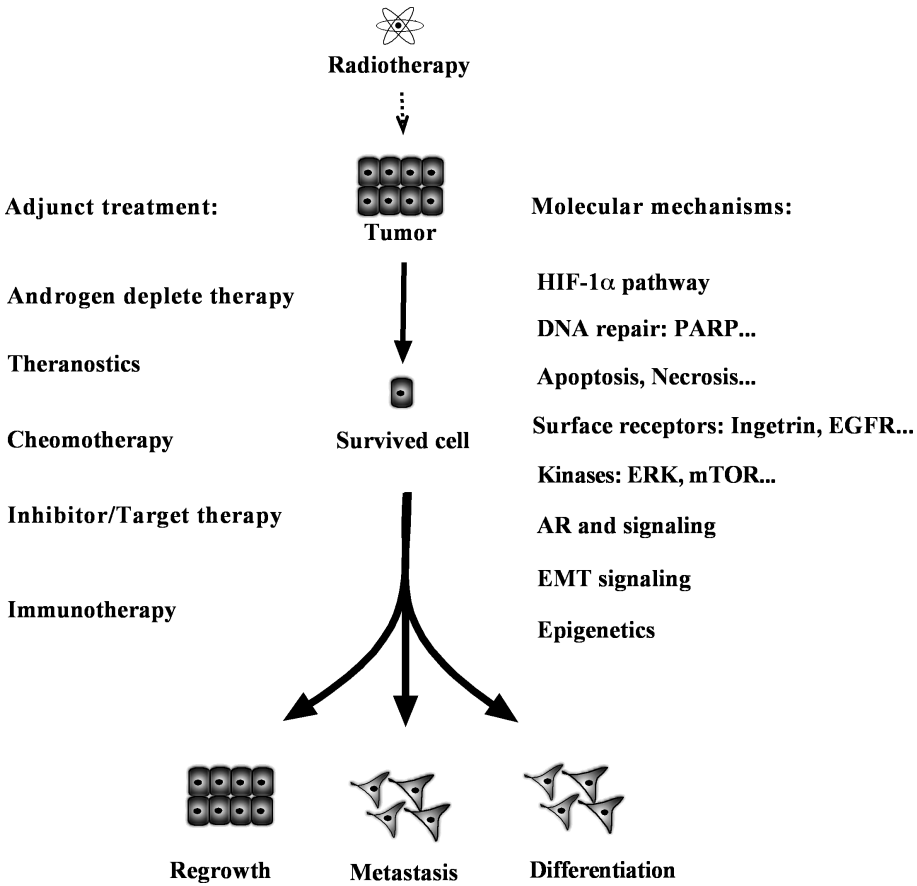

Figure 1. Therapeutic strategies associated with molecular mechanisms of prostate cancer cell survival upon ionizing radiation. Cells less sensitive to therapy will survive after being treated with radiation therapy. The schematic chart suggests the possible molecular mechanisms associated with tumor cell survival. With the modern development of imaging tools, hormone ablation therapy, target therapy, chemotherapy, immunotherapy and radiopharmacy, effective therapeutic strategies will evolve for the treatment of high-risk patients.

CONCLUSION

Molecular science of prostate cancer has matured at a rapid rate and direct applications of our growing knowledge are now on the horizon. Application of protocols to modulate cell adhesions, signaling pathways, survival pathways, epigenetic, DNA repair and hypoxia conditions or immunotherapy may prove to be important adjuncts in the clinical RT management of these patients (Figure 1). The success of therapies for low and intermediate risk may permit titration of

therapy adjusted to the sequelae of current management. For the high-risk patient, advances in our understanding of genomics/proteomics coupled with better understanding of molecular science will serve to improve outcome of high-risk patients affected with disease.

Conflict of Interest: The authors declare no potential conflicts of interest with respect to research, authorship and/or publication of this chapter.

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REFERENCES

1. Siegel RL, Miller KD, Jemal A. Cancer statistics, 2020. CA Cancer J Clin. 2020;70:7–30. https://doi.org/10.3322/caac.21590
2. Rosenthal SA, Hu C, Sartor O, Gomella LG, Amin MB, Purdy J, et al. Effect of Chemotherapy With Docetaxel With Androgen Suppression and Radiotherapy for Localized High-Risk Prostate Cancer: The Randomized Phase III NRG Oncology RTOG 0521 Trial. J Clin Oncol. 2019;37:1159–68. https://doi.org/10.1200/JCO.18.02158
3. Spratt DE, Zhang J, Santiago-Jimenez M, Dess RT, Davis JW, Den RB, et al. Development and Validation of a Novel Integrated Clinical-Genomic Risk Group Classification for Localized Prostate Cancer. J Clin Oncol. 2018;36:581–90. https://doi.org/10.1200/JCO.2017.74.2940
4. Nguyen PL, Martin NE, Choeurng V, Palmer-Aronsten B, Kolisnik T, Beard CJ, et al. Utilization of biopsy-based genomic classifier to predict distant metastasis after definitive radiation and short-course ADT for intermediate and high-risk prostate cancer. Prostate Cancer Prostatic Dis. 2017;20:186–92. https://doi.org/10.1038/pcan.2016.58
5. Shipley WU, Seiferheld W, Lukka HR, Major PP, Heney NM, Grignon DJ, et al. Radiation with or without Antiandrogen Therapy in Recurrent Prostate Cancer. N Engl J Med. 2017;376:417–28. https://doi.org/10.1056/NEJMoa1607529
6. Tosoian JJ, Birer SR, Jeffrey Karnes R, Zhang J, Davicioni E, Klein EE, et al. Performance of clinicopathologic models in men with high risk localized prostate cancer: impact of a 22-gene genomic classifier. Prostate Cancer Prostatic Dis. 2020;23:646–53. https://doi.org/10.1038/s41391-020-0226-2
7. Mohler JL, Armstrong AJ, Bahnson RR, D'Amico AV, Davis BJ, Eastham JA, et al. Prostate Cancer, Version 1.2016. J Natl Compr Canc Netw. 2016;14:19–30.
8. Gleason DF, Group. TVUR. Histologic grading and staging of prostatic carcinoma. In: Tannenbaum M, editor. Urologic Pathology. Philadelphia, PA: Lea & Febiger; 1997. p. 171–87.
9. Edge SB, Byrd DR, Compton CC, Fritz AG, Greene FL, Trotti A. AJCC Cancer Staging Manual. 7th ed. New York, NY: Springer; 2010.
10. Cooperberg MR, Hilton JF, Carroll PR. The CAPRA-S score: A straightforward tool for improved prediction of outcomes after radical prostatectomy. Cancer. 2011;117:5039–46. https://doi.org/10.1002/cncr.26169
11. Mahler C, Verhelst J, Denis L. Clinical pharmacokinetics of the antiandrogens and their efficacy in prostate cancer. Clin Pharmacokinet. 1998;34:405–17. https://doi.org/10.2165/00003088-199834050-00005
12. Machtens S, Schultheiss D, Kuczyk M, Truss MC, Jonas U. The history of endocrine therapy of benign and malignant diseases of the prostate. World J Urol. 2000;18:222–6. https://doi.org/10.1007/s003450000120

13. Granfors T, Modig H, Damber JE, Tomic R. Long-term followup of a randomized study of locally advanced prostate cancer treated with combined orchiectomy and external radiotherapy versus radiotherapy alone. J Urol. 2006;176:544–7. https://doi.org/10.1016/j.juro.2006.03.092
14. Saigal CS, Gore JL, Krupski TL, Hanley J, Schonlau M, Litwin MS, et al. Androgen deprivation therapy increases cardiovascular morbidity in men with prostate cancer. Cancer. 2007;110:1493–500. https://doi.org/10.1002/cncr.22933
15. Maillefert JF, Sibilia J, Michel F, Saussine C, Javier RM, Tavernier C. Bone mineral density in men treated with synthetic gonadotropin-releasing hormone agonists for prostatic carcinoma. J Urol. 1999;161:1219–22. https://doi.org/10.1016/S0022-5347(01)61639-2
16. Saylor PJ, Smith MR. Adverse effects of androgen deprivation therapy: defining the problem and promoting health among men with prostate cancer. J Natl Compr Canc Netw. 2010;8:211–23. https://doi.org/10.6004/jnccn.2010.0014
17. Wo JY, Zietman AL. Why does androgen deprivation enhance the results of radiation therapy? Urol Oncol. 2008;26:522–9. https://doi.org/10.1016/j.urolonc.2008.03.008
18. Schmidt-Hansen M, Hoskin P, Kirkbride P, Hasler E, Bromham N. Hormone and radiotherapy versus hormone or radiotherapy alone for non-metastatic prostate cancer: a systematic review with meta-analyses. Clin Oncol (R Coll Radiol). 2014;26:e21–46. https://doi.org/10.1016/j.clon.2014.06.016
19. Garcia-Barros M, Paris F, Cordon-Cardo C, Lyden D, Rafii S, Haimovitz-Friedman A, et al. Tumor response to radiotherapy regulated by endothelial cell apoptosis. Science. 2003;300:1155–9. https://doi.org/10.1126/science.1082504
20. Bergers G, Javaherian K, Lo KM, Folkman J, Hanahan D. Effects of angiogenesis inhibitors on multistage carcinogenesis in mice. Science. 1999;284:808–12. https://doi.org/10.1126/science.284.5415.808
21. Jimenez JA, Kao C, Raikwar S, Gardner TA. Current status of anti-angiogenesis therapy for prostate cancer. Urol Oncol. 2006;24:260–8. https://doi.org/10.1016/j.urolonc.2005.11.022
22. Lee EC, Zhan P, Schallhom R, Packman K, Tenniswood M. Antiandrogen-induced cell death in LNCaP human prostate cancer cells. Cell Death Differ. 2003;10:761–71. https://doi.org/10.1038/sj.cdd.4401228
23. Wang T, Alavian MR, Goel HL, Languino LR, Fitzgerald TJ. Bicalutamide inhibits androgen-mediated adhesion of prostate cancer cells exposed to ionizing radiation. Prostate. 2008;68:1734–42. https://doi.org/10.1002/pros.20838
24. Wang T, Huang J, Vue M, Alavian MR, Goel HL, Altieri DC, et al. alphavbeta3 Integrin Mediates Radioresistance of Prostate Cancer Cells Through Regulation of Survivin. Mol Cancer Res. 2018. https://doi.org/10.1158/1541-7786.MCR-18-0544
25. Goel HL, Sayeed A, Breen M, Zarif MJ, Garlick DS, Leav I, et al. beta1 integrins mediate resistance to ionizing radiation in vivo by inhibiting c-Jun amino terminal kinase 1. J Cell Physiol. 2013;228:1601–9. https://doi.org/10.1002/jcp.24323
26. Eke I, Makinde AY, Aryankalayil MJ, Reedy JL, Citrin DE, Chopra S, et al. Long-term Tumor Adaptation after Radiotherapy: Therapeutic Implications for Targeting Integrins in Prostate Cancer. Mol Cancer Res. 2018;16:1855–64. https://doi.org/10.1158/1541-7786.MCR-18-0232
27. Edelman MJ, Watson D, Wang X, Morrison C, Kratzke RA, Jewell S, et al. Eicosanoid modulation in advanced lung cancer: cyclooxygenase-2 expression is a positive predictive factor for celecoxib + chemotherapy--Cancer and Leukemia Group B Trial 30203. J Clin Oncol. 2008;26:848–55. https://doi.org/10.1200/JCO.2007.13.8081
28. Midgley RS, McConkey CC, Johnstone EC, Dunn JA, Smith JL, Grumett SA, et al. Phase III randomized trial assessing rofecoxib in the adjuvant setting of colorectal cancer: final results of the VICTOR trial. J Clin Oncol. 2010;28:4575–80. https://doi.org/10.1200/JCO.2010.29.6244
29. Wang T, Languino LR, Lian J, Stein G, Blute M, Fitzgerald TJ. Molecular targets for radiation oncology in prostate cancer. Front Oncol. 2011;1:17. https://doi.org/10.3389/fonc.2011.00017
30. Lu H, Wang T, Li J, Fedele C, Liu Q, Zhang J, et al. alphavbeta6 Integrin Promotes Castrate-Resistant Prostate Cancer through JNK1-Mediated Activation of Androgen Receptor. Cancer Res. 2016;76:5163–74. https://doi.org/10.1158/0008-5472.CAN-16-0543
31. Teoh M, Clark CH, Wood K, Whitaker S, Nisbet A. Volumetric modulated arc therapy: a review of current literature and clinical use in practice. Br J Radiol. 2011;84:967–96. https://doi.org/10.1259/bjr/22373346

32. van Herk M. Errors and margins in radiotherapy. Semin Radiat Oncol. 2004;14:52–64. https://doi.org/10.1053/j.semradonc.2003.10.003
33. Jaffray D, Kupelian P, Djemil T, Macklis RM. Review of image-guided radiation therapy. Expert Rev Anticancer Ther. 2007;7:89–103. https://doi.org/10.1586/14737140.7.1.89
34. Craig J, Woulfe J, Sinclair J, Malone S. Isolated brain metastases as first site of recurrence in prostate cancer: case report and review of the literature. Curr Oncol. 2015;22:e493–7. https://doi.org/10.3747/co.22.2542
35. Kume H, Takai K, Kameyama S, Kawabe K. Multiple pulmonary metastasis of prostatic carcinoma with little or no bone or lymph node metastasis. Report of two cases and review of the literature. Urol Int. 1999;62:44–7. https://doi.org/10.1159/000030355
36. Reinstatler L, Dupuis J, Dillon JL, Black CC, Phillips JD, Hyams ES. Lung malignancy in prostate cancer: A report of both metastatic and primary lung lesions. Urol Case Rep. 2018;16:119–22. https://doi.org/10.1016/j.eucr.2017.11.027
37. Pouessel D, Gallet B, Bibeau F, Avances C, Iborra F, Senesse P, et al. Liver metastases in prostate carcinoma: clinical characteristics and outcome. BJU Int. 2007;99:807–11. https://doi.org/10.1111/j.1464-410X.2006.06663.x
38. Jin JK, Dayyani F, Gallick GE. Steps in prostate cancer progression that lead to bone metastasis. Int J Cancer. 2011;128:2545–61. https://doi.org/10.1002/ijc.26024
39. Clarke NW, Hart CA, Brown MD. Molecular mechanisms of metastasis in prostate cancer. Asian J Androl. 2009;11:57–67. https://doi.org/10.1038/aja.2008.29
40. Heron PW, Abellan-Flos M, Salmon L, Sygusch J. Bisphosphonate Inhibitors of Mammalian Glycolytic Aldolase. J Med Chem. 2018;61:10558–72. https://doi.org/10.1021/acs.jmedchem.8b01000
41. Chandrasekar T, Yang JC, Gao AC, Evans CP. Mechanisms of resistance in castration-resistant prostate cancer (CRPC). Transl Androl Urol. 2015;4:365–80.
42. Spencer JA, Watson JM, Lubahn DB, Joseph DR, French FS, Wilson EM, et al. The androgen receptor gene is located on a highly conserved region of the X chromosomes of marsupial and monotreme as well as eutherian mammals. J Hered. 1991;82:134–9. https://doi.org/10.1093/oxfordjournals.jhered.a111047
43. Edwards J, Krishna NS, Grigor KM, Bartlett JM. Androgen receptor gene amplification and protein expression in hormone refractory prostate cancer. Br J Cancer. 2003;89:552–6. https://doi.org/10.1038/sj.bjc.6601127
44. Gioeli D, Ficarro SB, Kwiek JJ, Aaronson D, Hancock M, Catling AD, et al. Androgen receptor phosphorylation. Regulation and identification of the phosphorylation sites. J Biol Chem. 2002;277:29304–14. https://doi.org/10.1074/jbc.M204131200
45. Bhandari V, Hoey C, Liu LY, Lalonde E, Ray J, Livingstone J, et al. Molecular landmarks of tumor hypoxia across cancer types. Nat Genet. 2019;51:308–18. https://doi.org/10.1038/s41588-018-0318-2
46. Al-Ubaidi FL, Schultz N, Egevad L, Granfors T, Helleday T. Castration therapy of prostate cancer results in downregulation of HIF-1alpha levels. Int J Radiat Oncol Biol Phys. 2012;82:1243–8. https://doi.org/10.1016/j.ijrobp.2011.10.038
47. Inoue T, Ogawa O. Role of signaling transduction pathways in development of castration-resistant prostate cancer. Prostate Cancer. 2011;2011:647987. https://doi.org/10.1155/2011/647987
48. Nickols NG, Nazarian R, Zhao SG, Tan V, Uzunangelov V, Xia Z, et al. MEK-ERK signaling is a therapeutic target in metastatic castration resistant prostate cancer. Prostate Cancer Prostatic Dis. 2019;22:531–8. https://doi.org/10.1038/s41391-019-0134-5
49. Rodriguez-Berriguete G, Fraile B, Martinez-Onsurbe P, Olmedilla G, Paniagua R, Royuela M. MAP Kinases and Prostate Cancer. J Signal Transduct. 2012;2012:169170. https://doi.org/10.1155/2012/169170
50. Ciccarelli C, Di Rocco A, Gravina GL, Mauro A, Festuccia C, Del Fattore A, et al. Disruption of MEK/ERK/c-Myc signaling radiosensitizes prostate cancer cells in vitro and in vivo. J Cancer Res Clin Oncol. 2018;144:1685–99. https://doi.org/10.1007/s00432-018-2696-3
51. Wang T, Carraway RE, LeRoche D, FitzGerald TJ. Disruption of ERK1/2 sensitizes radiation resistance prostate cancer cells to paclitaxel and ionizing radiation. International J of Radiation Oncology, Biology and Physics. 2014;90:S806. https://doi.org/10.1016/j.ijrobp.2014.05.2326

52. Huang J, Yao JL, di Sant'Agnese PA, Yang Q, Bourne PA, Na Y. Immunohistochemical characterization of neuroendocrine cells in prostate cancer. Prostate. 2006;66:1399–406. https://doi.org/10.1002/pros.20434
53. Davies A, Zoubeidi A, Selth LA. The epigenetic and transcriptional landscape of neuroendocrine prostate cancer. Endocr Relat Cancer. 2020;27:R35-R50. https://doi.org/10.1530/ERC-19-0420
54. Krauss DJ, Hayek S, Amin M, Ye H, Kestin LL, Zadora S, et al. Prognostic significance of neuroendocrine differentiation in patients with Gleason score 8–10 prostate cancer treated with primary radiotherapy. Int J Radiat Oncol Biol Phys. 2011;81:e119–25. https://doi.org/10.1016/j.ijrobp.2010.12.064
55. Bonkhoff H. Neuroendocrine differentiation in human prostate cancer. Morphogenesis, proliferation and androgen receptor status. Ann Oncol. 2001;12 Suppl 2:S141–4. https://doi.org/10.1093/annonc/12.suppl_2.S141
56. Mosca A, Berruti A, Russo L, Torta M, Dogliotti L. The neuroendocrine phenotype in prostate cancer: basic and clinical aspects. J Endocrinol Invest. 2005;28:141–5.
57. Nelson EC, Cambio AJ, Yang JC, Ok JH, Lara PN, Jr., Evans CP. Clinical implications of neuroendocrine differentiation in prostate cancer. Prostate Cancer Prostatic Dis. 2007;10:6–14. https://doi.org/10.1038/sj.pcan.4500922
58. Yuan TC, Veeramani S, Lin MF. Neuroendocrine-like prostate cancer cells: neuroendocrine transdifferentiation of prostate adenocarcinoma cells. Endocr Relat Cancer. 2007;14:531–47. https://doi.org/10.1677/ERC-07-0061
59. Martin-Orozco RM, Almaraz-Pro C, Rodriguez-Ubreva FJ, Cortes MA, Ropero S, Colomer R, et al. EGF prevents the neuroendocrine differentiation of LNCaP cells induced by serum deprivation: the modulator role of PI3K/Akt. Neoplasia. 2007;9:614–24. https://doi.org/10.1593/neo.07337
60. Conteduca V, Burgio SL, Menna C, Carretta E, Rossi L, Bianchi E, et al. Chromogranin A is a potential prognostic marker in prostate cancer patients treated with enzalutamide. Prostate. 2014;74:1691–6. https://doi.org/10.1002/pros.22890
61. Berruti A, Mosca A, Tucci M, Terrone C, Torta M, Tarabuzzi R, et al. Independent prognostic role of circulating chromogranin A in prostate cancer patients with hormone-refractory disease. Endocr Relat Cancer. 2005;12:109–17. https://doi.org/10.1677/erc.1.00876
62. Sciarra A, Gentile V, Monti S, Mariotti G, Pastore A, Di Silverio F. Variation of chromogranin A serum levels after radical retropubic prostatectomy for prostate adenocarcinoma. Arch Androl. 2003;49:39–48. https://doi.org/10.1080/01485010390129160
63. Sarkar D, Singh SK, Mandal AK, Agarwal MM, Mete UK, Kumar S, et al. Plasma chromogranin A: clinical implications in patients with castrate resistant prostate cancer receiving docetaxel chemotherapy. Cancer Biomark. 2010;8:81–7. https://doi.org/10.3233/CBM-2011-0198
64. Deng X, Elzey BD, Poulson JM, Morrison WB, Ko SC, Hahn NM, et al. Ionizing radiation induces neuroendocrine differentiation of prostate cancer cells in vitro, in vivo and in prostate cancer patients. Am J Cancer Res. 2011;1:834–44.
65. Vashchenko N, Abrahamsson PA. Neuroendocrine differentiation in prostate cancer: implications for new treatment modalities. Eur Urol. 2005;47:147–55. https://doi.org/10.1016/j.eururo.2004.09.007
66. Wang T, Carraway RE, Briggs J, Chen H, FitzGerald TJ. Down-regulation of Bcl2 sensitizes radiation resistance prostate cancer cells to ionizing radiation. Program and Proceedings in Molecular Targets and Cancer Therapeutics. 2013;AACR-NCI-EORTC International Conference, Boston, MA:345. https://doi.org/10.1158/1535-7163.TARG-13-C50
67. Ellisen LW. PARP inhibitors in cancer therapy: promise, progress, and puzzles. Cancer Cell. 2011;19:165–7. https://doi.org/10.1016/j.ccr.2011.01.047
68. Pitroda SP, Pashtan IM, Logan HL, Budke B, Darga TE, Weichselbaum RR, et al. DNA repair pathway gene expression score correlates with repair proficiency and tumor sensitivity to chemotherapy. Sci Transl Med. 2014;6:229ra42. https://doi.org/10.1126/scitranslmed.3008291
69. Lord CJ, Ashworth A. The DNA damage response and cancer therapy. Nature. 2012;481:287–94. https://doi.org/10.1038/nature10760
70. Castro E, Mateo J, Olmos D, de Bono JS. Targeting DNA Repair: The Role of PARP Inhibition in the Treatment of Castration-Resistant Prostate Cancer. Cancer J. 2016;22:353–6. https://doi.org/10.1097/PPO.0000000000000219

71. Mateo J, Carreira S, Sandhu S, Miranda S, Mossop H, Perez-Lopez R, et al. DNA-Repair Defects and Olaparib in Metastatic Prostate Cancer. N Engl J Med. 2015;373:1697–708. https://doi.org/10.1056/NEJMoa1506859
72. Wang G, Zhao D, Spring DJ, DePinho RA. Genetics and biology of prostate cancer. Genes Dev. 2018;32:1105–40. https://doi.org/10.1101/gad.315739.118
73. Lloyd RL, Wijnhoven PWG, Ramos-Montoya A, Wilson Z, Illuzzi G, Falenta K, et al. Combined PARP and ATR inhibition potentiates genome instability and cell death in ATM-deficient cancer cells. Oncogene. 2020;39:4869–83. https://doi.org/10.1038/s41388-020-1328-y
74. Farmer H, McCabe N, Lord CJ, Tutt AN, Johnson DA, Richardson TB, et al. Targeting the DNA repair defect in BRCA mutant cells as a therapeutic strategy. Nature. 2005;434:917–21. https://doi.org/10.1038/nature03445
75. Asim M, Tarish F, Zecchini HI, Sanjiv K, Gelali E, Massie CE, et al. Synthetic lethality between androgen receptor signalling and the PARP pathway in prostate cancer. Nat Commun. 2017;8:374. https://doi.org/10.1038/s41467-017-00393-y
76. Christenson ES, Antonarakis ES. PARP inhibitors for homologous recombination-deficient prostate cancer. Expert Opin Emerg Drugs. 2018;23:123–33. https://doi.org/10.1080/14728214.2018.1459563
77. Hiew K, Hart CA, Ali A, Elliott T, Ramani V, Sangar V, et al. Primary Mutational Landscape Linked with Pre-Docetaxel Lactate Dehydrogenase Levels Predicts Docetaxel Response in Metastatic Castrate-Resistant Prostate Cancer. Eur Urol Focus. 2019;5:831–41. https://doi.org/10.1016/j.euf.2018.04.006
78. Hussain M, Mateo J, Fizazi K, Saad F, Shore N, Sandhu S, et al. Survival with Olaparib in Metastatic Castration-Resistant Prostate Cancer. N Engl J Med. 2020;383:2345–57. https://doi.org/10.1056/NEJMoa2022485
79. Kawalec P, Paszulewicz A, Holko P, Pilc A. Sipuleucel-T immunotherapy for castration-resistant prostate cancer. A systematic review and meta-analysis. Arch Med Sci. 2012;8:767–75. https://doi.org/10.5114/aoms.2012.31610
80. Kantoff PW, Higano CS, Shore ND, Berger ER, Small EJ, Penson DF, et al. Sipuleucel-T immunotherapy for castration-resistant prostate cancer. N Engl J Med. 2010;363:411–22. https://doi.org/10.1056/NEJMoa1001294
81. Gorchakov AA, Kulemzin SV, Kochneva GV, Taranin AV. Challenges and Prospects of Chimeric Antigen Receptor T-cell Therapy for Metastatic Prostate Cancer. Eur Urol. 2020;77:299–308. https://doi.org/10.1016/j.eururo.2019.08.014
82. Sharma P, Pachynski RK, Narayan V, Flechon A, Gravis G, Galsky MD, et al. Nivolumab Plus Ipilimumab for Metastatic Castration-Resistant Prostate Cancer: Preliminary Analysis of Patients in the CheckMate 650 Trial. Cancer Cell. 2020;38:489–99 e3. https://doi.org/10.1016/j.ccell.2020.08.007
83. Vikas P, Borcherding N, Chennamadhavuni A, Garje R. Therapeutic Potential of Combining PARP Inhibitor and Immunotherapy in Solid Tumors. Front Oncol. 2020;10:570. https://doi.org/10.3389/fonc.2020.00570
84. Garcia AJ, Ruscetti M, Arenzana TL, Tran LM, Bianci-Frias D, Sybert E, et al. Pten null prostate epithelium promotes localized myeloid-derived suppressor cell expansion and immune suppression during tumor initiation and progression. Mol Cell Biol. 2014;34:2017–28. https://doi.org/10.1128/MCB.00090-14
85. Han HJ, Li YR, Roach M, 3rd, Aggarwal R. Dramatic response to combination pembrolizumab and radiation in metastatic castration resistant prostate cancer. Ther Adv Med Oncol. 2020;12:1758835920936084. https://doi.org/10.1177/1758835920936084
86. Marks L, Young S, Natarajan S. MRI-ultrasound fusion for guidance of targeted prostate biopsy. Curr Opin Urol. 2013;23:43–50. https://doi.org/10.1097/MOU.0b013e32835ad3ee
87. Songmen S, Nepal P, Olsavsky T, Sapire J. Axumin Positron Emission Tomography: Novel Agent for Prostate Cancer Biochemical Recurrence. J Clin Imaging Sci. 2019;9:49. https://doi.org/10.25259/JCIS_139_2019
88. Kabasakal L, Demirci E, Nematyazar J, Akyel R, Razavi B, Ocak M, et al. The role of PSMA PET/CT imaging in restaging of prostate cancer patients with low prostate-specific antigen levels. Nucl Med Commun. 2017;38:149–55. https://doi.org/10.1097/MNM.0000000000000617

89. Crawford ED, Petrylak DP, Shore N, Saad F, Slovin SF, Vogelzang NJ, et al. The Role of Therapeutic Layering in Optimizing Treatment for Patients With Castration-resistant Prostate Cancer (Prostate Cancer Radiographic Assessments for Detection of Advanced Recurrence II). Urology. 2017;104:150–9. https://doi.org/10.1016/j.urology.2016.12.033
90. Crawford ED, Koo PJ, Shore N, Slovin SF, Concepcion RS, Freedland SJ, et al. A Clinician's Guide to Next Generation Imaging in Patients With Advanced Prostate Cancer (RADAR III). J Urol. 2019;201:682–92. https://doi.org/10.1016/j.juro.2018.05.164
91. El-Amm J, Aragon-Ching JB. Radium-223 for the treatment of castration-resistant prostate cancer. Onco Targets Ther. 2015;8:1103–9. https://doi.org/10.2147/OTT.S44291
92. Jones W, Griffiths K, Barata PC, Paller CJ. PSMA Theranostics: Review of the Current Status of PSMA-Targeted Imaging and Radioligand Therapy. Cancers (Basel). 2020;12. https://doi.org/10.3390/cancers12061367
93. Cook GJ, Azad G, Padhani AR. Bone imaging in prostate cancer: the evolving roles of nuclear medicine and radiology. Clin Transl Imaging. 2016;4:439–47. https://doi.org/10.1007/s40336-016-0196-5
94. Yu Z, Carlucci G, Ananias HJ, Dierckx RA, Liu S, Helfrich W, et al. Evaluation of a technetium-99m labeled bombesin homodimer for GRPR imaging in prostate cancer. Amino Acids. 2013;44:543–53. https://doi.org/10.1007/s00726-012-1369-9
95. Wong KK, Piert M. Dynamic bone imaging with 99mTc-labeled diphosphonates and 18F-NaF: mechanisms and applications. J Nucl Med. 2013;54:590–9. https://doi.org/10.2967/jnumed.112.114298
96. Filippi L, Basile P, Schillaci O, Bagni O. The Relationship Between Total Lesion Activity on (18)F Choline Positron Emission Tomography-Computed Tomography and Clinical Outcome in Patients with Castration-Resistant Prostate Cancer Bone Metastases Treated with (223)Radium. Cancer Biother Radiopharm. 2020;35:398–403. https://doi.org/10.1089/cbr.2019.3188
97. Wallitt KL, Khan SR, Dubash S, Tam HH, Khan S, Barwick TD. Clinical PET Imaging in Prostate Cancer. Radiographics. 2017;37:1512–36. https://doi.org/10.1148/rg.2017170035
98. Almeida FD, Yen CK, Scholz MC, Lam RY, Turner J, Bans LL, et al. Performance characteristics and relationship of PSA value/kinetics on carbon-11 acetate PET/CT imaging in biochemical relapse of prostate cancer. Am J Nucl Med Mol Imaging. 2017;7:1–11.
99. Heck MM, Tauber R, Schwaiger S, Retz M, D'Alessandria C, Maurer T, et al. Treatment Outcome, Toxicity, and Predictive Factors for Radioligand Therapy with (177)Lu-PSMA-I&T in Metastatic Castration-resistant Prostate Cancer. Eur Urol. 2019;75:920–6. https://doi.org/10.1016/j.eururo.2018.11.016
100. Morgenstern A, Apostolidis C, Kratochwil C, Sathekge M, Krolicki L, Bruchertseifer F. An Overview of Targeted Alpha Therapy with (225)Actinium and (213)Bismuth. Curr Radiopharm. 2018;11:200–8. https://doi.org/10.2174/1874471011666180502104524

8

Revisiting the Role of p53 in Prostate Cancer

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Doi: https://doi.org/10.36255/exonpublications.prostatecancer.p53.2021

Abstract: Mutations in the tumor suppressor gene *TP53* are among the most common genetic aberrations in cancer. In prostate cancer, the role of mutant *TP53* remains incompletely understood. Initially, mutations in *TP53* were considered late events during malignant progression and associated with metastatic dissemination and castration resistance. However, recent studies report an inactivation of *TP53* at an unexpectedly high frequency in primary as well as metastatic castration-naïve prostate cancer. In this chapter, we discuss the biology of p53, the relevance of *TP53* mutations for prostate cancer progression and therapy

In: *Prostate Cancer*. Bott SRJ, Ng KL (Editors). Exon Publications, Brisbane, Australia. ISBN: 978-0-6450017-5-4; Doi: https://doi.org/10.36255/exonpublications.prostatecancer.2021

resistance, and its potential role as a marker to identify patients who require more intensified treatment.

Keywords: castration-naïve prostate cancer; castration-resistant prostate cancer; p53, therapy resistance; *TP53*

INTRODUCTION

Prostate cancer is the most common non-cutaneous cancer in men (1). Due to the introduction of broader screening and testing for prostate-specific antigen (PSA) blood levels, the majority of prostate cancers are now diagnosed at a localized state (2). Prostate cancer is a heterogeneous disease, and the clinical outcome of localized prostate cancer is highly variable. Approximately 30% of men suffer from relapse despite definitive local treatment by radical prostatectomy or percutaneous radiotherapy (3). Localized prostate carcinoma already shows a substantial molecular and genetic diversity (4). There is hence an urgent clinical need to identify molecular and genetic markers with predictive and prognostic relevance in addition to "classical" outcome parameters such as TNM stage, Gleason score and initial PSA level (5). A better characterization of genetic factors associated with more aggressive tumor growth kinetics could influence clinical decision-making with respect to more personalized neoadjuvant and/or adjuvant strategies (6).

The finding that a significant proportion of men with advanced prostate cancer harbor germline and/or somatic mutations in DNA damage repair genes has been a major advancement in the management of the disease (7). It has been known for a while that tumors with DNA damage repair gene defects are associated with earlier metastatic dissemination and poorer disease outcome (8–11). At the same time, mutations, in particular in *BRCA1* and *BRCA2,* create a therapeutic vulnerability that has been exploited by the use of PARP inhibitors in patients with metastatic, castration-resistant prostate cancer (mCRPC) (11, 12). Recent results from several phase II and III trials confirm a clinical advantage of PARP inhibition in terms of progression-free and overall survival (12–18). However, there is mounting evidence that not all patients who are broadly categorized as carrying DNA damage repair gene defects (in fact many of these genes play only indirect roles in DNA damage repair) benefit from PARP inhibition (19). Therefore, additional molecular markers are needed to characterize therapeutic vulnerabilities, treatment resistance and patient prognosis with an even higher resolution.

A gene that is typically not included in targeted next-generation sequencing (NGS) panels used in key phase II and III trials to identify patients for PARP inhibitor treatment is *TP53*, one of the most frequently altered tumor suppressor genes in human cancer.

THE EVOLUTION AND FUNCTION OF p53

p53 was first discovered in 1979 and initially thought to be an oncogene (20–24). Subsequent work demonstrated that the transcription factor p53, together with

its E3 ubiquitin ligase MDM2, is at the center of a signaling node that plays a crucial role in stress response and tissue homeostasis (25). Over hundreds of millions of years, the p53 family has evolved from protecting the germline of invertebrates from mutations to a more general signaling hub that preserves the tissue integrity of vertebrates (25). p53 responds to a diverse array of cellular stresses by activating the transcription of genes that either lead to a reconstitution of the damaged cell or its elimination by apoptosis or cellular senescence (26). p53-dependent transcription hence promotes cell cycle arrest, DNA repair, metabolic adaptation, or the upregulation of pro-apoptotic genes such as *BAX* or *PUMA* or pro-senescence genes such as *PML* or *CDKN1A*. These properties as key regulator of cell fate decision make p53 the single most critical human tumor suppressor and contribute to the fact that *TP53* is the most commonly altered gene in human cancer (27, 28).

Physiologically, p53 is expressed at a low level in most normal cells, which involves a number of cellular antagonists, most importantly its E3 ubiquitin ligase MDM2 and its heterodimerization partner, MDM4 (29). By ubiquitinating p53, MDM2 drives the proteasomal degradation of p53 (30). MDM2 itself is positively regulated by p53 thus creating a feedback loop to ensure low p53 protein levels in the absence of cellular stress. Over a dozen of extrinsic and intrinsic stress signals have been reported to feed into the MDM2-p53 signaling node to cause activation of p53-dependent gene transcription (25). The p53 response is activated by decreased degradation upon disruption of the p53/MDM2/MDM4 complex leading to p53 stabilization. The disruption of these interactions is regulated by posttranslational modifications of MDM2 and/or p53 such as phosphorylation by protein kinases activated by stress such as ATR, ATM, CHK1, CHK2 or DNA-PK, among others (25, 31). Additional mechanisms of p53 activation exist such as the nucleolar sequestration of MDM2 by ARF in response to oncogene stress (32). Another mechanism of activation of the MDM2-p53 node involves the deubiquitinating enzyme HAUSP (33). Obviously, different sources of cellular stress can trigger distinct modes of p53 activation depending on the responding protein kinases.

Upon its activation, p53 binds to the promoter of p53-responsive target genes to activate gene transcription. MDM2 and MDM4 are co-recruited to these promoters where they form a complex with p53 to modulate target gene activation (34).

STRUCTURE OF p53

The tumor suppressor gene *TP53* encodes a protein with 393 amino acids and is located on chromosome 17p13.1 (35). The p53 protein comprises an N-terminal transactivation domain, a proline-rich domain, a central DNA-binding domain, followed by a tetramerization domain and an intrinsically disordered C-terminal regulatory domain (36). Inactivating mutations in *TP53* occur in approximately 50% of human cancers, and mutation rates range between more than 90% and below 5% depending on the tumor type (37). Most mutations are detected in the central DNA-binding domain, thereby incapacitating the function of p53 as a transcription factor. Missense mutations, frameshift deletions and frameshift

insertions account for approximately 70% of pathogenic mutations (37). Inactivation of both *TP53* alleles is found in over 90% of cancers with *TP53* mutations, most commonly through a single missense mutation and loss of the second allele through a deletion of chromosome 17p (37). Missense mutations frequently lead to an impaired degradation by MDM2 thus stabilizing the protein and rendering it easily detectable as overexpressed by immunohistochemistry (38). Remarkably, the top hotspot missense mutations occur at methylated CpG sites, which encode evolutionary conserved arginine residues. The most common mutation is R175H, followed by R248Q, R273H, R248W, R273C, and R282W, which account for approximately a quarter of all *TP53* missense mutations (39). As a functional consequence of these mutations, the transcriptional activation of p53-specific target genes is disrupted (40, 41) although gain-of-function mutations have also been described (42).

In addition to acquired mutations, germline mutations of *TP53* have been identified in patients with Li-Fraumeni syndrome. The Li-Fraumeni syndrome is characterized by sarcomas, breast and adrenal cortex carcinomas, cerebral tumors, and acute leukemias at a young age (43, 44). Germline mutations in *TP53* are highly penetrant with an up to 100% cumulative lifetime risk to develop cancer (45).

TP53 MUTATIONS IN PROSTATE CANCER

Initially, inactivation of *TP53* has been suggested to be a late event during prostate cancer progression (46–49). While it is now firmly established that mCRPC has the highest *TP53* mutations rates (see below), there is emerging evidence that *TP53* mutations can also be found at a relatively high frequency in primary, and, especially, in castration-naïve metastatic prostate cancer (50–56).

In the TCGA cohort, whole genome sequencing of 333 samples from men with localized prostate cancer was performed and a mutation rate in *TP53* of 8% was detected (51). In a different study, sequencing of 111 cases of primary prostate cancer revealed a *TP53* mutation rate of 6% (57).

Remarkably, the rate of *TP53* mutations in castration-naïve metastatic prostate cancer was between 28% and 36% and hence significantly higher than in primary prostate cancer (50, 52, 58) and only exceeded by mutation rates found in mCRPC. Analysis of 150 mCRPC samples showed a *TP53* mutation rate of 53% (59). In additional studies, the *TP53* mutation rate was between 31% and 73% (53, 60–63). Whole-exome sequencing data from 410 mCRPCs identified 33% of tumors with a biallelic loss of *TP53* and 32% with single-copy loss or a pathogenic mutation (62). These findings confirm the marked differences in the *TP53* mutation rate in primary, metastatic castration-naïve and castration-resistant prostate cancer.

Important insights into the role of *TP53* deficiency in disease progression stem from studies that incorporate patient outcome measurements and longitudinal studies. Hamid and colleagues showed that *TP53* alterations increase from localized castration-naïve prostate cancer (20%) to metastatic castration-naïve prostate cancer (37%) and mCRPC (73%) and are associated with an approximately 2-fold risk for disease recurrence in patients with primary prostate cancer (53). In a recent

study by Mateo and colleagues, primary prostate cancer specimens from 175 patients who later developed mCRPC were analyzed. Mutations and homozygous loss of *TP53* were the most frequently detected aberrations and found in 25% of the primary tumors (52). In addition, there appears to be an increase of *TP53* alterations, besides alterations of the androgen receptor (AR) pathway, when same-patient specimens obtained from the untreated primary tumor and mCRPC were compared (52).

In conclusion, there is emerging evidence for a high rate of *TP53* mutations in primary prostate cancer predisposed to a lethal disease outcome as well as prostate cancer with metastatic dissemination at the time of diagnosis.

p53 AND RESISTANCE OF PROSTATE CANCER TO SYSTEMIC THERAPY

Prostate cancer growth and progression exquisitely depends on androgens, and androgen deprivation still remains the most important treatment modality for patients with recurrent or metastatic disease (64). However, all patients ultimately develop tumor progression and castration resistance (65). The role of *TP53* inactivation in response to androgen deprivation therapy has not been studied in detail. Thus far, there appears to be no negative impact of *TP53* alterations in the response to first-line antihormonal treatment (52). In the last decade, several novel therapeutic options for patients with mCRPC have been established including the CYP17 inhibitor abiraterone and the androgen receptor antagonist enzalutamide (66, 67). Since not all men benefit from these next-generation antiandrogens, there is a clinical need for markers that indicate primary or acquired resistance to aid decision-making. Because mCRPC still critically depends on AR signaling (68), the constitutively active AR splice variant V7 (AR-V7) has been suggested as a crucial, albeit not exclusive, component of the resistance mechanisms to next-generation antiandrogens (69, 70). De Laere and colleagues could demonstrate that inactivation of *TP53* was associated with significantly shorter progression-free and overall survival of prostate cancer patients treated with abiraterone or enzalutamide (71). The poorest progression free survival was found in patients with a biallelic *TP53* inactivation. Of note, *TP53* mutations were the only marker independently associated with an unfavorable response to abiraterone and enzalutamide and, remarkably, outperformed genomic AR alterations and expression of AR splice variants (71). How p53 influences resistance to next-generation antiandrogens remains to be clarified. Interestingly, there is evidence to suggest that wild-type p53 may suppress AR activation (72–74).

The microtubule-stabilizing agent docetaxel is the only chemotherapy that has been shown to extend survival in patients with mCRPC (75, 76). The response of prostate cancer cells to docetaxel has been found to be compromised by mutant p53 (77). The clinical utility of *TP53* mutation status as a predictive marker for docetaxel treatment hence warrants further investigation.

Whether and to what extend *TP53* perturbations affect the response to the PARP inhibitor olaparib, which has recently been approved for patients with mCRPC and *BRCA1/2* mutations (16, 18), is currently unclear.

TP53 AND THE CLONAL EVOLUTION OF PROSTATE CANCER

Since a substantial proportion of primary prostate cancers harbor mutations in *TP53*, the question arises whether *TP53* inactivation may be a driver event for malignant progression. There is mounting evidence that this could be the case. *TP53* mutations have been reported as truncal aberrations in considerable proportions of metastatic prostate cancers (58, 78). Interestingly, a case study could demonstrate that a mutant *TP53* clone originating from a small, well-differentiated focus of primary prostate cancer was apparently the origin of metastatic spread with a 17-year lag period (79). However, *TP53* mutations have also been reported to be enriched in metastatic lesions and there are also examples of tumors in which *TP53* aberrations can be found exclusively in metastases (52, 80).

In conclusion, *TP53* mutations seem to be an early event in some prostate cancers while in others an enrichment in metastatic lesions can be found. In the future, increasingly sensitive detection methods such as single-cell sequencing hold the promise to even better define the molecular composition of primary and metastatic prostate cancer with respect to the *TP53* mutation status.

DOES p53 HAVE POTENTIAL AS A THERAPEUTIC TARGET AFTER ALL?

Given the high frequency of *TP53* inactivation in prostate cancer and in cancer in general, the question remains how this finding could be translated into a therapeutic vulnerability. p53 is notoriously difficult to target and numerous studies have used approaches such as gene therapy, inhibition of MDM2 or MDM4 interactions, synthetic lethal approaches, and others (81–85). It should not be forgotten that p53 has originally been discovered as a tumor antigen induced by chemical carcinogens (86). Hence, approaches to exploit mutant p53 as immunological target as well as the increased genomic instability of p53-defective cells through immune oncological interventions still appear promising. In this context, an exacerbation of the mutational burden may further enhance the therapeutic vulnerability of p53-deficient cells to promote responses to immune checkpoint inhibitors.

CONCLUSION

Inactivation of *TP53* has initially been described as a late event during malignant progression and associated mainly with mCRPC. There is now compelling evidence that mutated *TP53* can also be detected in primary prostate cancer, and, especially, in castration-naïve metastatic prostate cancer. Inactivation of *TP53* predicts an unfavorable patient outcome, early metastatic dissemination, and resistance to next-generation antiandrogens. Therefore, *TP53* perturbations have a strong potential as a marker to identify patients with a high risk for lethal disease outcome who could benefit from more intensified treatment.

Conflict of interest: The authors declare no potential conflict of interest with respect to research, authorship and/or publication of this chapter

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REFERENCES

1. Siegel RL, Miller KD, Jemal A. Cancer statistics, 2020. CA Cancer J Clin. 2020;70(1):7–30. https://doi.org/10.3322/caac.21590
2. Schröder FH, Hugosson J, Roobol MJ, Tammela TL, Ciatto S, Nelen V, et al. Screening and prostate-cancer mortality in a randomized European study. N Engl J Med. 2009;360(13):1320–8. https://doi.org/10.1056/NEJMoa0810084
3. Paller CJ, Antonarakis ES. Management of biochemically recurrent prostate cancer after local therapy: evolving standards of care and new directions. Clin Adv Hematol Oncol. 2013;11(1):14–23.
4. Boutros PC, Fraser M, Harding NJ, de Borja R, Trudel D, Lalonde E, et al. Spatial genomic heterogeneity within localized, multifocal prostate cancer. Nat Genet. 2015;47(7):736–45. https://doi.org/10.1038/ng.3315
5. D'Amico AV. Risk-based management of prostate cancer. N Engl J Med. 2011;365(2):169–71. https://doi.org/10.1056/NEJMe1103829
6. Beltran H, Rubin MA. New strategies in prostate cancer: translating genomics into the clinic. Clin Cancer Res. 2013;19(3):517–23. https://doi.org/10.1158/1078-0432.CCR-12-1452
7. Mateo J, Boysen G, Barbieri CE, Bryant HE, Castro E, Nelson PS, et al. DNA repair in prostate cancer: biology and clinical implications. Eur Urol. 2017;71(3):417–25. https://doi.org/10.1016/j.eururo.2016.08.037
8. Bednarz N, Eltze E, Semjonow A, Rink M, Andreas A, Mulder L, et al. BRCA1 loss preexisting in small subpopulations of prostate cancer is associated with advanced disease and metastatic spread to lymph nodes and peripheral blood. Clin Cancer Res. 2010;16(13):3340–8. https://doi.org/10.1158/1078-0432.CCR-10-0150
9. Castro E, Jugurnauth-Little S, Karlsson Q, Al-Shahrour F, Piñeiro-Yañez E, Van de Poll F, et al. High burden of copy number alterations and c-MYC amplification in prostate cancer from BRCA2 germline mutation carriers. Ann Oncol. 2015;26(11):2293–300. https://doi.org/10.1093/annonc/mdv356
10. Castro E, Goh C, Leongamornlert D, Saunders E, Tymrakiewicz M, Dadaev T, et al. Effect of BRCA mutations on metastatic relapse and cause-specific survival after radical treatment for localised prostate cancer. Eur Urol. 2015;68(2):186–93. https://doi.org/10.1016/j.eururo.2014.10.022
11. Castro E, Goh C, Olmos D, Saunders E, Leongamornlert D, Tymrakiewicz M, et al. Germline BRCA mutations are associated with higher risk of nodal involvement, distant metastasis, and poor survival outcomes in prostate cancer. J Clin Oncol. 2013;31(14):1748–57. https://doi.org/10.1200/JCO.2012.43.1882
12. Mateo J, Carreira S, Sandhu S, Miranda S, Mossop H, Perez-Lopez R, et al. DNA-repair defects and olaparib in metastatic prostate cancer. N Engl J Med. 2015;373(18):1697–708. https://doi.org/10.1056/NEJMoa1506859
13. Mateo J, Porta N, Bianchini D, McGovern U, Elliott T, Jones R, et al. Olaparib in patients with metastatic castration-resistant prostate cancer with DNA repair gene aberrations (TOPARP-B): a multicentre, open-label, randomised, phase 2 trial. Lancet Oncol. 2020;21(1):162–74. https://doi.org/10.1016/S1470-2045(19)30684-9
14. Abida W, Bryce AH, Vogelzang NJ, Amatao R, Percent I, Shapiro J. Preliminary results from TRITON2: a phase 2 study of rucaparib in patients with metastatic castration-resistant prostate cancer (mCRPC)

associated with homologous recombination repair (HRR) gene alterations. Ann Oncol. 2018;29 (suppl 8):vii271-vii302. https://doi.org/10.1093/annonc/mdy284.002
15. Smith M, Sandhu S, Kelly W, Scher H, Efstathiou E, Lara P, et al. Pre-specified interim analysis of GALAHAD: a phase II study of niraparib in patients (pts) with metastatic castration-resistant prostate cancer (mCRPC) and biallelic DNA-repair gene defects (DRD). Ann Oncol. 2019;30:v884-v5. https://doi.org/10.1093/annonc/mdz394.043
16. Hussain M, Mateo J, Fizazi K, Saad F, Shore N, Sandhu S, et al. PROfound: Phase III study of olaparib versus enzalutamide or abiraterone for metastatic castration-resistant prostate cancer (mCRPC) with homologous recombination repair (HRR) gene alterations. Ann Oncol. 2019;30:v881-v2. https://doi.org/10.1093/annonc/mdz394.039
17. Hussain M, Mateo J, Fizazi K, Saad F, Shore N, Sandhu S, et al. Survival with olaparib in metastatic castration-resistant prostate cancer. N Engl J Med. 2020;383(24):2345–57. https://doi.org/10.1056/NEJMoa2022485
18. de Bono J, Mateo J, Fizazi K, Saad F, Shore N, Sandhu S, et al. Olaparib for metastatic castration-resistant prostate cancer. N Engl J Med. 2020;382(22):2091–102. https://doi.org/10.1056/NEJMoa1911440
19. Markowski MC, Antonarakis ES. BRCA1 versus BRCA2 and PARP inhibitor sensitivity in prostate cancer: more different than alike? J Clin Oncol. 2020;38(32):3735–9. https://doi.org/10.1200/JCO.20.02246
20. Vogelstein B, Lane D, Levine AJ. Surfing the p53 network. Nature. 2000;408(6810):307–10. https://doi.org/10.1038/35042675
21. DeLeo AB, Jay G, Appella E, Dubois GC, Law LW, Old LJ. Detection of a transformation-related antigen in chemically induced sarcomas and other transformed cells of the mouse. Proc Natl Acad Sci U S A. 1979;76(5):2420–4. https://doi.org/10.1073/pnas.76.5.2420
22. Lane DP, Crawford LV. T antigen is bound to a host protein in SY40-transformed cells. Nature. 1979;278(5701):261–3. https://doi.org/10.1038/278261a0
23. Linzer DI, Levine AJ. Characterization of a 54K dalton cellular SV40 tumor antigen present in SV40-transformed cells and uninfected embryonal carcinoma cells. Cell. 1979;17(1):43–52. https://doi.org/10.1016/0092-8674(79)90293-9
24. Mercer WE, Avignolo C, Baserga R. Role of the p53 protein in cell proliferation as studied by microinjection of monoclonal antibodies. Mol Cell Biol. 1984;4(2):276–81. https://doi.org/10.1128/MCB.4.2.276
25. Levine AJ. p53: 800 million years of evolution and 40 years of discovery. Nat Rev Cancer. 2020;20(8):471–80. https://doi.org/10.1038/s41568-020-0262-1
26. Lane DP. p53, guardian of the genome. Nature. 1992;358(6381):15–6. https://doi.org/10.1038/358015a0
27. Levine AJ, Momand J, Finlay CA. The p53 tumour suppressor gene. Nature. 1991;351(6326):453–6. https://doi.org/10.1038/351453a0
28. Oren M. p53: the ultimate tumor suppressor gene? Faseb J. 1992;6(13):3169–76. https://doi.org/10.1096/fasebj.6.13.1397838
29. Liu Y, Tavana O, Gu W. p53 modifications: exquisite decorations of the powerful guardian. J Mol Cell Biol. 2019;11(7):564–77. https://doi.org/10.1093/jmcb/mjz060
30. Oliner JD, Pietenpol JA, Thiagalingam S, Gyuris J, Kinzler KW, Vogelstein B. Oncoprotein MDM2 conceals the activation domain of tumour suppressor p53. Nature. 1993;362(6423):857–60. https://doi.org/10.1038/362857a0
31. Durocher D, Jackson SP. DNA-PK, ATM and ATR as sensors of DNA damage: variations on a theme? Curr Opin Cell Biol. 2001;13(2):225–31. https://doi.org/10.1016/S0955-0674(00)00201-5
32. Weber JD, Taylor LJ, Roussel MF, Sherr CJ, Bar-Sagi D. Nucleolar Arf sequesters Mdm2 and activates p53. Nat Cell Biol. 1999;1(1):20–6. https://doi.org/10.1038/8991
33. Li M, Chen D, Shiloh A, Luo J, Nikolaev AY, Qin J, et al. Deubiquitination of p53 by HAUSP is an important pathway for p53 stabilization. Nature. 2002;416(6881):648–53. https://doi.org/10.1038/nature737
34. Marine JC, Francoz S, Maetens M, Wahl G, Toledo F, Lozano G. Keeping p53 in check: essential and synergistic functions of Mdm2 and Mdm4. Cell Death Differ. 2006;13(6):927–34. https://doi.org/10.1038/sj.cdd.4401912

35. Mertens F, Johansson B, Höglund M, Mitelman F. Chromosomal imbalance maps of malignant solid tumors: a cytogenetic survey of 3185 neoplasms. Cancer Res. 1997;57(13):2765–80.
36. el-Deiry WS, Kern SE, Pietenpol JA, Kinzler KW, Vogelstein B. Definition of a consensus binding site for p53. Nat Genet. 1992;1(1):45–9. https://doi.org/10.1038/ng0492-45
37. Donehower LA, Soussi T, Korkut A, Liu Y, Schultz A, Cardenas M, et al. Integrated analysis of TP53 gene and pathway alterations in the cancer genome atlas. Cell Rep. 2019;28(5):1370–84.e5. https://doi.org/10.1016/j.celrep.2019.07.001
38. Rodrigues NR, Rowan A, Smith ME, Kerr IB, Bodmer WF, Gannon JV, et al. p53 mutations in colorectal cancer. Proc Natl Acad Sci U S A. 1990;87(19):7555–9. https://doi.org/10.1073/pnas.87.19.7555
39. Baugh EH, Ke H, Levine AJ, Bonneau RA, Chan CS. Why are there hotspot mutations in the TP53 gene in human cancers? Cell Death Differ. 2018;25(1):154–60. https://doi.org/10.1038/cdd.2017.180
40. Olivier M, Hollstein M, Hainaut P. TP53 mutations in human cancers: origins, consequences, and clinical use. Cold Spring Harb Perspect Biol. 2010;2(1):a001008-a. https://doi.org/10.1101/cshperspect.a001008
41. de Vries A, Flores ER, Miranda B, Hsieh HM, van Oostrom CT, Sage J, et al. Targeted point mutations of p53 lead to dominant-negative inhibition of wild-type p53 function. Proc Natl Acad Sci U S A. 2002;99(5):2948–53. https://doi.org/10.1073/pnas.052713099
42. Oren M, Rotter V. Mutant p53 gain-of-function in cancer. Cold Spring Harb Perspect Biol. 2010;2(2):a001107. https://doi.org/10.1101/cshperspect.a001107
43. Li FP, Fraumeni JF, Jr. Soft-tissue sarcomas, breast cancer, and other neoplasms. A familial syndrome? Ann Intern Med. 1969;71(4):747–52. https://doi.org/10.7326/0003-4819-71-4-747
44. Birch JM, Alston RD, McNally RJQ, Evans DGR, Kelsey AM, Harris M, et al. Relative frequency and morphology of cancers in carriers of germline TP53 mutations. Oncogene. 2001;20(34):4621–8. https://doi.org/10.1038/sj.onc.1204621
45. Malkin D. Li-Fraumeni syndrome. Genes Cancer. 2011;2(4):475–84. https://doi.org/10.1177/1947601911413466
46. Navone NM, Troncoso P, Pisters LL, Goodrow TL, Palmer JL, Nichols WW, et al. p53 protein accumulation and gene mutation in the progression of human prostate carcinoma. J Natl Cancer Inst. 1993;85(20):1657–69. https://doi.org/10.1093/jnci/85.20.1657
47. Heidenberg HB, Sesterhenn IA, Gaddipati JP, Weghorst CM, Buzard GS, Moul JW, et al. Alteration of the tumor suppressor gene p53 in a high fraction of hormone refractory prostate cancer. J Urol. 1995;154(2 Pt 1):414–21. https://doi.org/10.1016/S0022-5347(01)67065-4
48. Hall MC, Navone NM, Troncoso P, Pollack A, Zagars GK, von Eschenbach AC, et al. Frequency and characterization of p53 mutations in clinically localized prostate cancer. Urology. 1995;45(3):470–5. https://doi.org/10.1016/S0090-4295(99)80018-1
49. Bookstein R, MacGrogan D, Hilsenbeck SG, Sharkey F, Allred DC. p53 is mutated in a subset of advanced-stage prostate cancers. Cancer Res. 1993;53(14):3369–73.
50. Nientiedt C, Endris V, Jenzer M, Mansour J, Sedehi NTP, Pecqueux C, et al. High prevalence of DNA damage repair gene defects and TP53 alterations in men with treatment-naïve metastatic prostate cancer-results from a prospective pilot study using a 37 gene panel. Urol Oncol. 2020;38(7):637.e17-.e27. https://doi.org/10.1016/j.urolonc.2020.03.001
51. Abeshouse A, Ahn J, Akbani R, Ally A, Amin S, Andry Christopher D, et al. The molecular taxonomy of primary prostate cancer. Cell. 2015;163(4):1011–25. https://doi.org/10.1016/j.cell.2015.10.025
52. Mateo J, Seed G, Bertan C, Rescigno P, Dolling D, Figueiredo I, et al. Genomics of lethal prostate cancer at diagnosis and castration resistance. J Clin Invest. 2020;130(4):1743–51. https://doi.org/10.1172/JCI132031
53. Hamid AA, Gray KP, Shaw G, MacConaill LE, Evan C, Bernard B, et al. Compound genomic alterations of TP53, PTEN, and RB1 tumor suppressors in localized and metastatic prostate cancer. Eur Urol. 2019;76(1):89–97. https://doi.org/10.1016/j.eururo.2018.11.045
54. Liu W, Xie CC, Thomas CY, Kim S-T, Lindberg J, Egevad L, et al. Genetic markers associated with early cancer-specific mortality following prostatectomy. Cancer. 2013;119(13):2405–12. https://doi.org/10.1002/cncr.27954

55. Fraser M, Sabelnykova VY, Yamaguchi TN, Heisler LE, Livingstone J, Huang V, et al. Genomic hallmarks of localized, non-indolent prostate cancer. Nature. 2017;541(7637):359–64. https://doi.org/10.1038/nature20788
56. Armenia J, Wankowicz SAM, Liu D, Gao J, Kundra R, Reznik E, et al. The long tail of oncogenic drivers in prostate cancer. Nat Genet. 2018;50(5):645–51. https://doi.org/10.1038/s41588-018-0078-z
57. Barbieri CE, Baca SC, Lawrence MS, Demichelis F, Blattner M, Theurillat J-P, et al. Exome sequencing identifies recurrent SPOP, FOXA1 and MED12 mutations in prostate cancer. Nat Genet. 2012;44(6):685–9. https://doi.org/10.1038/ng.2279
58. Abida W, Armenia J, Gopalan A, Brennan R, Walsh M, Barron D, et al. Prospective genomic profiling of prostate cancer across disease states reveals germline and somatic alterations that may affect clinical decision making. JCO Precis Oncol. 2017;2017:PO.17.00029.
59. Robinson D, Van Allen EM, Wu YM, Schultz N, Lonigro RJ, Mosquera JM, et al. Integrative clinical genomics of advanced prostate cancer. Cell. 2015;161(5):1215–28. https://doi.org/10.1016/j.cell.2015.05.001
60. Beltran H, Yelensky R, Frampton GM, Park K, Downing SR, MacDonald TY, et al. Targeted next-generation sequencing of advanced prostate cancer identifies potential therapeutic targets and disease heterogeneity. Eur Urol. 2013;63(5):920–6. https://doi.org/10.1016/j.eururo.2012.08.053
61. Kumar A, White TA, MacKenzie AP, Clegg N, Lee C, Dumpit RF, et al. Exome sequencing identifies a spectrum of mutation frequencies in advanced and lethal prostate cancers. Proc Natl Acad Sci U S A. 2011;108(41):17087–92. https://doi.org/10.1073/pnas.1108745108
62. Nyquist MD, Corella A, Coleman I, De Sarkar N, Kaipainen A, Ha G, et al. Combined TP53 and RB1 loss promotes prostate cancer resistance to a spectrum of therapeutics and confers vulnerability to replication stress. Cell Rep. 2020;31(8):107669. https://doi.org/10.1016/j.celrep.2020.107669
63. van Dessel LF, van Riet J, Smits M, Zhu Y, Hamberg P, van der Heijden MS, et al. The genomic landscape of metastatic castration-resistant prostate cancers reveals multiple distinct genotypes with potential clinical impact. Nat Commun. 2019;10(1):5251. https://doi.org/10.1038/s41467-019-13084-7
64. Sartor O, de Bono JS. Metastatic prostate cancer. N Engl J Med. 2018;378(7):645–57. https://doi.org/10.1056/NEJMra1701695
65. Attard G, de Bono JS. Translating scientific advancement into clinical benefit for castration-resistant prostate cancer patients. Clin Cancer Res. 2011;17(12):3867–75. https://doi.org/10.1158/1078-0432.CCR-11-0943
66. de Bono JS, Logothetis CJ, Molina A, Fizazi K, North S, Chu L, et al. Abiraterone and increased survival in metastatic prostate cancer. N Engl J Med. 2011;364(21):1995–2005. https://doi.org/10.1056/NEJMoa1014618
67. Scher HI, Fizazi K, Saad F, Taplin ME, Sternberg CN, Miller K, et al. Increased survival with enzalutamide in prostate cancer after chemotherapy. N Engl J Med. 2012;367(13):1187–97. https://doi.org/10.1056/NEJMoa1207506
68. Attard G, Parker C, Eeles RA, Schröder F, Tomlins SA, Tannock I, et al. Prostate cancer. Lancet. 2016;387(10013):70–82. https://doi.org/10.1016/S0140-6736(14)61947-4
69. Antonarakis ES, Lu C, Wang H, Luber B, Nakazawa M, Roeser JC, et al. AR-V7 and resistance to enzalutamide and abiraterone in prostate cancer. N Engl J Med. 2014;371(11):1028–38. https://doi.org/10.1056/NEJMoa1315815
70. Bernemann C, Schnoeller TJ, Luedeke M, Steinestel K, Boegemann M, Schrader AJ, et al. Expression of AR-V7 in circulating tumour cells does not preclude response to next generation androgen deprivation therapy in patients with castration resistant prostate cancer. Eur Urol. 2017;71(1):1–3. https://doi.org/10.1016/j.eururo.2016.07.021
71. De Laere B, Oeyen S, Mayrhofer M, Whitington T, van Dam PJ, Van Oyen P, et al. TP53 outperforms other androgen receptor biomarkers to predict abiraterone or enzalutamide outcome in metastatic castration-resistant prostate cancer. Clin Cancer Res. 2019;25(6):1766–73. https://doi.org/10.1158/1078-0432.CCR-18-1943
72. Shenk JL, Fisher CJ, Chen SY, Zhou XF, Tillman K, Shemshedini L. p53 represses androgen-induced transactivation of prostate-specific antigen by disrupting hAR amino- to carboxyl-terminal interaction. J Biol Chem. 2001;276(42):38472–9. https://doi.org/10.1074/jbc.M103652200

73. Gurova KV, Roklin OW, Krivokrysenko VI, Chumakov PM, Cohen MB, Feinstein E, et al. Expression of prostate specific antigen (PSA) is negatively regulated by p53. Oncogene. 2002;21(1):153–7. https://doi.org/10.1038/sj.onc.1205001
74. Cronauer MV, Schulz WA, Burchardt T, Ackermann R, Burchardt M. Inhibition of p53 function diminishes androgen receptor-mediated signaling in prostate cancer cell lines. Oncogene. 2004;23(20):3541–9. https://doi.org/10.1038/sj.onc.1207346
75. Tannock IF, de Wit R, Berry WR, Horti J, Pluzanska A, Chi KN, et al. Docetaxel plus prednisone or mitoxantrone plus prednisone for advanced prostate cancer. N Engl J Med. 2004;351(15):1502–12. https://doi.org/10.1056/NEJMoa040720
76. Petrylak DP. The treatment of hormone-refractory prostate cancer: docetaxel and beyond. Rev Urol. 2006;8(Suppl 2):S48-S55.
77. Liu C, Zhu Y, Lou W, Nadiminty N, Chen X, Zhou Q, et al. Functional p53 determines docetaxel sensitivity in prostate cancer cells. Prostate. 2013;73(4):418–27. https://doi.org/10.1002/pros.22583
78. Gundem G, Van Loo P, Kremeyer B, Alexandrov LB, Tubio JMC, Papaemmanuil E, et al. The evolutionary history of lethal metastatic prostate cancer. Nature. 2015;520(7547):353–7. https://doi.org/10.1038/nature14347
79. Haffner MC, Mosbruger T, Esopi DM, Fedor H, Heaphy CM, Walker DA, et al. Tracking the clonal origin of lethal prostate cancer. J Clin Invest. 2013;123(11):4918–22. https://doi.org/10.1172/JCI70354
80. Hong MK, Macintyre G, Wedge DC, Van Loo P, Patel K, Lunke S, et al. Tracking the origins and drivers of subclonal metastatic expansion in prostate cancer. Nat Commun. 2015;6:6605. https://doi.org/10.1038/ncomms7605
81. Eastham JA, Hall SJ, Sehgal I, Wang J, Timme TL, Yang G, et al. In vivo gene therapy with p53 or p21 adenovirus for prostate cancer. Cancer Res. 1995;55(22):5151–5.
82. Chappell WH, Lehmann BD, Terrian DM, Abrams SL, Steelman LS, McCubrey JA. p53 expression controls prostate cancer sensitivity to chemotherapy and the MDM2 inhibitor Nutlin-3. Cell Cycle. 2012;11(24):4579–88. https://doi.org/10.4161/cc.22852
83. Duffy MJ, Synnott NC, McGowan PM, Crown J, O'Connor D, Gallagher WM. p53 as a target for the treatment of cancer. Cancer Treat Rev. 2014;40(10):1153–60. https://doi.org/10.1016/j.ctrv.2014.10.004
84. Hong B, van den Heuvel AP, Prabhu VV, Zhang S, El-Deiry WS. Targeting tumor suppressor p53 for cancer therapy: strategies, challenges and opportunities. Curr Drug Targets. 2014;15(1):80–9. https://doi.org/10.2174/1389450114666140106101412
85. Sabapathy K, Lane DP. Therapeutic targeting of p53: all mutants are equal, but some mutants are more equal than others. Nat Rev Clin Oncol. 2018;15(1):13–30. https://doi.org/10.1038/nrclinonc.2017.151
86. DeLeo AB, Appella E. The p53 saga: early steps in the development of tumor immunotherapy. J Immunol. 2020;204(9):2321–8. https://doi.org/10.4049/jimmunol.1901343

9

MUCIN 1 in Prostate Cancer

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Doi: https://doi.org/10.36255/exonpublications.prostatecancer.mucin1.2021

Abstract: Despite extensive research efforts in prostate cancer for the last several decades, the disease remains a leading cause of cancer death in men in the developed world. A typical feature of prostate cancer initiation and progression is the landscape of genetic alterations, which changes the expression patterns of numerous molecules in prostate epithelial cells, where the disease originates. These aberrantly expressed proteins are tumor-associated antigens. Their uniqueness in tumors offers an avenue not only in advancing our understanding of prostate cancer but also in the search for better diagnostic and therapeutic tools. Mucin 1 is one of the most well-characterized tumor-associated antigens. The protein is overexpressed and aberrantly glycosylated following prostate cancer development, and influences certain disease factors including disease initiation, metastasis, and resistance to therapy. Mucin 1 possesses value as a biomarker in predicting prostate cancer prognosis and has been studied as a therapeutic target. This chapter provides an overview of the impact of Mucin 1 on prostate cancer and its clinical values.

Keywords: biomarkers; mucin 1; prostate cancer; prostate cancer vaccine; therapy resistance

In: *Prostate Cancer*. Bott SRJ, Ng KL (Editors). Exon Publications, Brisbane, Australia. ISBN: 978-0-6450017-5-4; Doi: https://doi.org/10.36255/exonpublications.prostatecancer.2021

INTRODUCTION

Prostate cancer remains the most prevalent malignancy and the second leading cause of cancer-related death in men in the developed world (1). The disease originates from the prostate epithelial cells as prostatic intra-epithelial neoplasia and progresses to invasive carcinoma and metastatic prostate cancer (2, 3). Metastases occur frequently in the bone (4). Primary prostate cancers are commonly managed by active surveillance, and curative treatments including radical prostatectomy and radiation. Approximately 30% of patients following radical prostatectomy will develop recurrent tumors or biochemical recurrence with rise in serum prostate-specific antigen (PSA) (5). Recurrent tumors are typically resistant to therapy, and relapsed prostate cancers or prostate cancers with resurged PSA are associated with higher risk of metastasis (6). Metastatic prostate cancers are treated with androgen deprivation therapy (ADT), which commonly leads to resistance in the form of castration-resistant prostate cancer (CRPC) (7, 8). There are multiple therapeutic options available for CRPCs, including taxane-based chemotherapy and those targeting androgen receptor signaling such as abiraterone or enzalutamide (8–10), and immunotherapy (11, 12). Despite this variety of treatment options, CRPC remains lethal (8, 13).

Cancer initiation, progression, and development of therapy resistance are regulated by complex processes, owing to the genetic and epigenetic changes that occur during the course of oncogenesis. These alterations result in a large number of unique tumor-associated antigens (TAAs) (14, 15). PSA as a classic prostate cancer TAA has been shown to generate PSA-specific T cells (16, 17). The nature of cancer-specific alterations (overexpression and modification) makes TAAs attractive targets for diagnostic and therapeutic purposes. Mucin 1 (MUC1) is one of the most well-characterized TAAs. MUC1 promotes tumorigenesis by activating PI3K-AKT, MEK-ERK, and other molecular pathways (18). Overexpression, hypoglycosylation, and aberrant glycosylation of MUC1 occur during prostate cancer initiation and progression. These changes are also associated with relapse and CRPC development. Thus, changes in MUC1 can be used as a prognostic biomarker. As a TAA, MUC1 has been explored as a target candidate for prostate cancer vaccine. This chapter provides an overview of the role of MUC1 in prostate cancer. The biology of MUC1, its alterations during prostate cancer development and progression, and its potential as a therapeutic target along with its limitations and future research are discussed.

THE BIOLOGY OF MUC1

The *MUC1* gene at 1q22 encodes mucin 1, a protein belonging to the 21-member mucin family in humans. Mucins are large proteins with extensive O-glycosylation and constitute the mucus barrier on epithelium to protect epithelial cells from external environment (19). MUC1 was first detected in human milk fat globule and a set of breast cancer cell lines using anti-human milk fat globule serum (anti-HMFG) (20); its membrane expression was subsequently observed at the apical surface of many glandular epithelial cells including those of the mammary gland, salivary gland, pancreas, prostate, uterus, as well as gastrointestinal and

respiratory tracts (21, 22). MUC1 plays a critical role in forming the protective mucus barrier on epithelial surfaces, evident by the significant reduction of mucus obstruction in cystic fibrosis mice with MUC1 deficiency (23).

Cell surface MUC1 is a heterodimer consisting of a large N-terminal extracellular subunit (MUC1-N or α-subunit) and a small C-terminal subunit (MUC1-C or β-subunit) containing a small extracellular domain, a transmembrane motif, and a C-terminal intracellular region; dimers are formed via non-covalent association in extracellular regions adjacent to cell membrane (Figure 1) (24). The two subunits are produced from a single polypeptide chain by autocleavage following the GSVVV sequence, which is located within the SEA (Sea urchin sperm protein enterokinase and agrin) domain, during translation (25). The N-terminal fragment contains variable number of tandem repeats (VNTR, n = 40–80) of 20 amino acid residues (26, 27); MUC-N is enriched with proline, threonine, and serine (PTS) motifs and is extensively O-glycosylated such that the peptide core is mostly covered (Figure 1) (28). The heavy glycosylation contributes to MUC1's physiological functions in normal cells (28).

In cancer cells, MUC1 is not only significantly upregulated but also undergoes aberrant glycosylation and hypoglycosylation in most cancers (29). Hypoglycosylation leads to exposure of VNTR peptides, which along with aberrant glycosylation change the biochemical properties and cell distributions of MUC1 (28). These abnormalities underline MUC1's properties as a biomarker and therapeutic target as well as its functionality in promoting cancer progression.

UPREGULATION OF *MUC1* IN PROSTATE CANCER

In a study of 2760 prostate cancer cases and 1722 controls, *MUC1* gene variations in terms of single nucleotide polymorphisms and haplotype were not associated

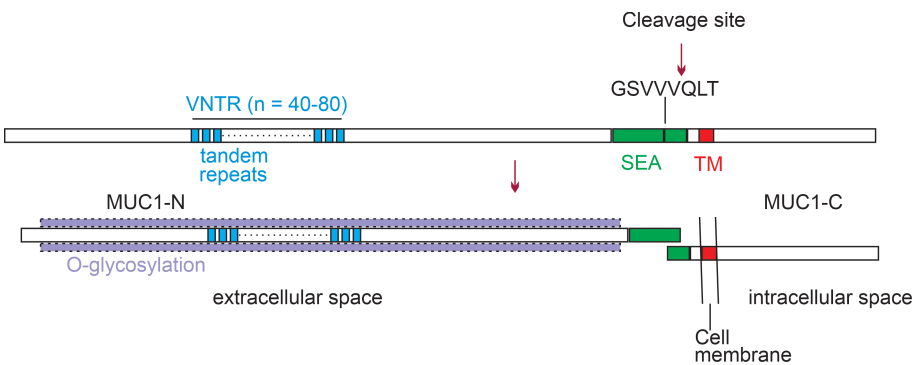

Figure 1. MUC1 heterodimer structure. MUC1 is cleaved at the indicated site, i.e., after GSVVV, during translation to generate the MUC1-N and MUC1-C subunits. Both subunits form a heterodimer in the extracellular space adjacent to cell membrane. MUC1-N is extensively O-glycosylated as indicated. SEA (Sea urchin sperm protein enterokinase and agrin) and TM (transmembrane) domains are indicated. VNTR, variable number of tandem repeats.

with prostate cancer risk and disease progression (30). In an investigation of primary prostate cancers (n = 333), metastatic prostate cancers (n = 150), and CRPCs (n = 77), an increase in *MUC1* gene copy number was observed in 35% of CRPCs compared to 6% and 1.8% in mPCs and primary PCs, respectively (31), indicating that *MUC1* gene amplification contributes to MUC1 upregulation in CRPCs.

In a NanoString-based gene expression analysis using 7 pairs of primary prostate cancers and matched non-tumor tissues, MUC1 mRNA was increased in four prostate cancer samples compared to their matched non-tumor controls; 5 of the PC tissues showed elevations of ERG expression (demonstrative of TMPRSS2-ERG fusion) and downregulation of PTEN, both common molecular alterations in prostate cancer oncogenesis (31). However, in an analysis of multiple cohorts consisting of 221 prostate cancers and 92 normal prostate tissues, MUC1 mRNA expression was shown to be reduced (31). Nonetheless, high level of MUC1 mRNA expression likely correlates with TMPRSS2-ERG fusion based on data from the Sueltman dataset (Figure 2A) (32). TMPRSS2-ERG fusion occurs commonly in prostate cancer and plays important roles in its initiation and progression (33, 34). Additionally, microarray-based gene expression profiling of 62 primary prostate cancers and 41 normal prostate tissues revealed increases in MUC1 mRNA expression in high-grade and advanced prostate cancers (35). Collectively, while current evidence does not conclusively support upregulation of MUC1 gene expression during prostate cancer initiation, elevations in MUC1 mRNA largely correlate with prostate cancer progression.

The above concept is supported by increases in MUC1 mRNA expression in metastatic prostate cancers. In two independent cohorts containing 54 metastatic prostate cancers compared to 82 normal prostate tissues, higher levels of MUC1 mRNA were observed in metastatic cases (31). Elevation of MUC1 mRNA in metastatic prostate cancer could also be demonstrated using the well-established Sawyers dataset (36) organized by the R2: Genomics Analysis and Visualization Platform (http://r2.amc.nl http://r2platform.com) (Figure 2B).

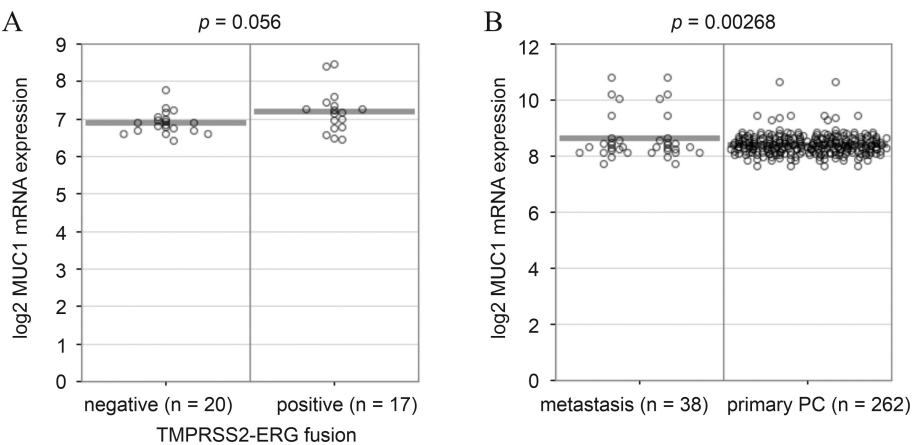

Figure 2. MUC1 expression is associated with adverse features of PC. A. Analyses were performed using the Sueltman dataset (45) in R2: Genomics Analysis and Visualization Platform. **B.** Analyses were performed using the Sawyers dataset (49) in R2: Genomics. Statistical analyses were performed by the R2 Platform using one-way ANOVA.

MUC1 expression was observed in prostate epithelial cells and prostate adenocarcinoma more than two decades ago using two anti-MUC1 monoclonal antibodies (mAb) DF3 and 139H2 (22). Immunohistochemistry staining with B27.29, which recognizes the peptide core (37), showed enhanced MUC1 protein expression in prostate cancer compared to normal prostate glandular epithelial cells (38). Hypoglycosylation of prostate cancer-associated MUC1 was demonstrated by its preferential recognition of prostate cancer cells compared to non-tumor prostate epithelial cells using antibodies BrE-3, BC2, and EMA; these mAbs bind to the peptide core. The upregulation of hypoglycosylated MUC1 positively correlates with Gleason scores (39) and cancer progression (40, 41) (Table 1).

Reduction in O-glycosylation in tumor-associated MUC1 is also caused by premature termination of chain elongation, which is in part attributed to the addition of sialic acid, leading to MUC1 being highly sialylated in tumors (28). In line with this concept, mAb MY.1E12 which reacts with sialylated MUC1 (42, 43), detects MUC1 upregulation and is correlated with prostate cancer grade (44). Elevation of 2 O-linked glycan syalyl Lewis X (sLe^x) MUC1 occurs in prostate cancer (Table 1), which might be in part attributable to the upregulation of GCNT1 glycosyltransferase in prostate cancer (45).

While evidence collectively supports overexpression of aberrantly glycosylated MUC1 in prostate cancer, it remains unclear whether the "upregulation" detected by antibodies recognizing the altered forms truly reflects MUC1 upregulation, as aberrantly modified MUC1 is present in prostate epithelial cells. This limitation is reflected in immunohistochemistry analysis using mAb MBC-2, which revealed MUC1 positivity in 28% of primary prostate cancers (9/32), and 22% of non-tumor prostate tissues (15/68) (46). Similarly, MUC1 protein was detected in 17%

TABLE 1 MUC1 upregulation in prostate cancer[a]

Population (n)[b]	MAb	% of positive	Association[c]	Reference
10	DF3 139H2	100% 100%	NA	(22)
5	B27.29	NA	NA	(38)
24	BrE-3, BC2, EMA	NA	Upregulation in PC and higher Gleason grade PC	(39)
120 primary PC 10 LN mPC	C595	58% 90%	Upregulation in PC and higher Gleason grade PC	(40)
9 mPC	HMFG-2	55.5%[d]	Upregulation in mPC	(41)
57	MY.1E12	NA	Upregulation in PC and higher Gleason grade PC	(44)
10	CHO131	NA	NA	(45)

[a]In comparison to normal prostate and/or BPH (benign prostate hyperplasia) tissues; [b]Primary PCs unless otherwise indicated; [c]Association with PC severity; [d] Positivity was defined by MUC1-positive cells > 50% of total tumor cells. LN mPC, lymph node metastasis; mPC, distant metastasis; NA, not available.

(30/175) of prostate cancer and 41% (42/103) of non-tumor tissues using the VNTR-specific, but glycosylation-insensitive, anti-MUC1 antibody 214D4 (47). It is thus important to further examine MUC1 upregulation using gene expression and genetic approaches.

While the mechanisms responsible for MUC1 upregulation in prostate cancer at either the protein or mRNA level are still largely unknown, prostate cancer stem cells (PCSC) may play a role in this process. Sphere cells derived from DU145 cells possess PCSC properties (48) and display significant upregulation of MUC1 at both the protein and mRNA level compared to their non-stem cancer counterparts (31). Higher levels of MUC1 were also detected in xenografts generated from DU145 PCSC-like sphere cells compared to tumors produced by non-stem cancer DU145 cells (31). Evidence indicates that mechanisms regulating PCSCs might be important in MUC1 upregulation in prostate cancer. This notion is in accordance with the expression of MUC1*, a MUC-1C fragment missing the N-terminal 13 residues from its 58 residues of the extracellular domain in human embryonic stem cells (hESCs) (49). PCSCs are a major driver of prostate cancer progression and development of therapy resistance, including CRPC (50).

MUC1 AND PROSTATE CANCER PROGRESSION

Resistance to ADT or the generation of CRPC remains the inevitable, lethal progression of prostate cancer, to which PCSC is a major contributor (50). Of note, upregulation of MUC1 has been demonstrated in human CRPCs, LNCaP cell-derived CRPC xenografts, and CRPC produced in castrated prostate-specific *PTEN*$^{-/-}$ mice (31, 51). MUC1 promotes CRPC in part via enhancement of PCSC. MUC1-C induces the expression of the pluripotent genes *OCT4*, *SOX2*, *LKF4*, and *MYC* in prostate cancer cells, facilitates PCSCs, and promotes CRPC development (52). Intriguingly, MUC1* maintains the self-renewal of hESCs via binding to NM23-H1, a metastasis-associated protein (49). MUC1-C enhances prostate cancer plasticity partly through suppression of AR signaling (52). MUC1-C reduces AR signaling via association with ARs and activating miR-135 that downregulates ARs (53). ARs downregulate MUC1 expression in LNCaP cells via binding to the *MUC1* promoter, and also through induction of miR-125b that inhibits MUC1 expression (54). The AR-derived suppression of MUC1 expression might be a contributor for LNCaP cells being MUC1-negative (55). While these observations support mutual inhibition between ARs and MUC1 expression in prostate cancer, their relationship is complex; ectopic expression of ARs in AR-negative PC3 cells upregulated MUC1 following stimulation with 5α-dihydrotestosterone (DHT) (56). Similar observations were also obtained in AR-negative DU145 cells with ectopic AR expression (57).

Induction of MUC1 by androgens in DU145-AR and PC3-AR cells decreased cell adhesion (56, 57). Upregulation of MUC1 in PC3 cells by arctiin also reduced cell adhesion (58), supporting the idea that MUC1 plays an important role in decreasing cell adhesion, which may facilitate metastasis. This possibility is reinforced by the production of sialyl Lewis a (sLe^{a}) modification on MUC1 upon its ectopic expression in low MUC1 expression LNCaP and PC3 cells (59).

MUC1 with the sLe^a and sLe^x antigen are selectin ligands (60–62); the interaction between cancer cells and selectin plays a critical role in the extravasation of cancer cells from blood vessel to tissues during metastasis (63). MUC1 may enhance metastasis via multiple mechanisms. For example, MUC1-C can induce the epithelial-mesenchymal transition (EMT) (53), an essential process of metastasis. MUC1 also enhances prostate cancer progression through other mechanisms. The inhibition of AMPKα activity by MUC1 *in vivo* promotes CRPC development; conversely, AMPKα suppresses CRPC in part by inhibition of MUC1 expression (64). While the detailed mechanisms are still unclear, MUC1 expression in prostate cancer is associated with angiogenesis (65) and evasion of natural killer cell-derived immunity (66). Downregulation of MUC1 expression by miR-326 inhibited cell proliferation *in vitro* and xenograft formation *in vivo*; the inhibitions were neutralized upon MUC1 re-expression (67). Collectively, a large body of evidence reveals that MUC1 plays a role in promoting prostate cancer progression through modulating multiple oncogenic processes, including angiogenesis, metastasis, and CRPC development. These properties might be attributed to MUC1-C's action in promoting growth factor receptor signaling, PI3K-AKT-mTOR, MEK-ERK, and cancer metabolism (18).

MUC1-MEDIATED PREDICTION OF PROSTATE CANCER PROGNOSIS

The upregulation of aberrant glycosylation along with its functional contributions to prostate cancer underlines MUC1's potential as a prognostic biomarker. MUC1 expression can be used for risk stratification (44), predicting tumor volume, stage, metastasis (68), recurrence-free survival (35, 69) and mortality risk (70). MUC1-mediated prediction of prostate cancer recurrence and fatality can be improved with multiple gene panels consisting of MUC1+AZGP1 (35) and MUC1+AZGP1+p53 (70), respectively. Furthermore, MUC1-associated genes or its network predicted prostate cancer relapse with high level of certainty (51, 71). Collectively, accumulative evidence supports an association of high MUC1 expression with poor prognosis of PC (Table 2).

Nonetheless, the prognostic role of MUC1 in prostate cancer might be much more complex. In a tissue microarray analysis of early-stage prostate cancer (T1a-b, Nx, M0; n = 195) under watchful waiting for 20 years, tumors with either high- or low-MUC1 expression were associated with a higher risk of fatality compared to those with moderate MUC1 expression comparable to normal prostate epithelium (72). MUC1's prognostic potential was independent of Gleason score and tumor stage (72). The observed higher risk of death for early-stage prostate cancers with reduced MUC1 expression needs further investigation. Nonetheless, this study indicates a complex relationship between MUC1 expression and prostate cancer progression, a concept that is in line with the observations that overexpression of MUC1 in LNCaP C4–2B4 cells was neither stimulative nor inhibitive of xenograft formation (73). Collectively, more work is needed to translate the knowledge generated in laboratory into clinical applications.

TABLE 2 **MUC1-associated prognostic biomarker value**

Population (n)	Progression	HR (95% CI)[a]	p value	Reference
57	PFS	5.23 (1.83-14.97	0.002**	(44)
225	RFS	2.35 (1.30-4.24)	0.0005***	(35)
119[b]	DSS	3.2 (1.5-7.0)[c]	0.0382*	(68)
1326	RFS	1.24 (1.02-1.49)	0.02*	(69)
315[d]	OS	2.51 (1.14-5.54)	0.02*	(70)
485[e]	DFS	2.38 (1.55-3.58)	3.45E-05***	(51)

[a]Univariate Cox analysis unless otherwise specified; [b]Patients with LN metastasis; [c]Multivariate Cox analysis including Gleason scores; [d]Mortality cases n = 83; [e] A nine-gene panel derived from MUC1-associated genes. DFS, disease free survival; DSS, disease-specific survival; OS, overall survival; PFS, progression free survival; RFS, recurrence free survival; *p<0.05, **p<0.01, and ***: p < 0.001.

MUC1 AS A THERAPEUTIC TARGET FOR PROSTATE CANCER

As a TAA, MUC1 has been examined as a target for immunotherapy for prostate cancer. In an *in vitro* model, chimeric antigen receptor (CAR)-MUC1 T cells were produced and shown to be effective in killing PC3 and DU145 cells; they also increased the cytotoxicity of AR-positive LNCaP cells together with flutamide, an anti-androgen (74). Tecemotide or L-BLP25 is a cancer vaccine targeting the tandem repeats of MUC1 and has been under clinical trials for a variety of cancers, including a phase III trial for non-small cell lung carcinoma (NSCLC) (75, 76). A phase II clinical trial has been conducted on 16 patients who had biochemical recurrence following radical prostatectomy. Of these, six patients showed prolonged PSA doubling time (PSADT) (77). In a phase I/II clinical trial (NCT00852007) on 17 patients with non-metastatic CRPC, autologous dendritic cells were stimulated with a Tn-MUC1 peptide *in vitro*, and upon reintroduction to patients, it significantly improved PSADT in 11 patients and induced Tn-MUC1 specific $CD4^+$ and $CD8^+$ T cell response in five of the seven patients analyzed (78). In a randomized phase IIa clinical trial on 21 chemonaïve CRPC patients with dendritic cells loaded with NY-ESO-1, MAGE-C2, and MUC1 peptides, specific T cell responses were detected and in patients with IFN-γ^+ T cells, extension of median radiological progression-free survival was observed (79).

MUC1 has also been targeted using a virus-based vaccine. TG4010 is a recombinant vaccinia virus Ankara expressing MUC1 and IL2. In a phase II clinical trial on 40 prostate cancer patients with PSA progression treated with TG4010, 13 patients had at least a 2-fold improvement in PSADT, and 10 patients had stabilized PSA for more than 8 months (80). Although the primary objective of a 50% PSA reduction from base line was not achieved, inclusion of

MUC1 in the vaccine provided some therapeutic benefits. Collectively, the above observations support MUC1 being a useful TAA for developing prostate cancer vaccine.

CONCLUSION

Since its discovery as a component of human milk fat globule in 1977 (20), MUC1 has been extensively studied in cancer, particularly in epithelium-originated malignancy; it is commonly overexpressed with aberrant glycosylation in numerous cancer types (19), including prostate cancer. Despite some inconsistencies (46), cumulative evidence clearly reveals MUC1 upregulation in prostate cancer, and its possible role in initiation, progression, and metastasis of prostate cancer. While MUC1 expression does show prognostic value, this prediction is not robust and should be strengthened by multigene panels for potential clinical application. In this regard, the multigene panels derived from MUC1's network (51, 71) should be explored for clinical applications. While MUC1 as a TAA has clinical benefits as a vaccine, its therapeutic potential seems limited based on several clinical trials in which MUC1 tandem repeat peptide core and aberrant glycosylation have been used. Approaches to inhibition of MUC1-C warrant more attention. Of note, GO-201, a synthetic peptide that inhibits MUC1-C oligomerization displays anti-prostate cancer activity in preclinical studies (81). Additionally, upon linkage to ZZ-PE38, the Fc-binding ZZ domain of protein A fused to *Pseudomonas* exotoxin (82), a humanized mAb DMB5F3 potently killed $MUC1^{+}$ cancer cells (83). DMB5F3 recognizes the SEA domain shared between MUC1-N and MUC1-C (83). The therapeutic utility of GO-201 and DMB5F3-ZZ-PE38 in treating prostate cancer should be investigated either alone or together with the current MUC1 vaccines. Further, the role of MUC1 on $MUC1^{-/-}$ mice and MUC1 transgenic animals should be investigated. Both mouse lines are available (84, 85). Transgenic expression of human MUC1 in mice did not cause tumor formation (85). $MUC1^{-/-}$ mice were normal (84) but showed delay in mammary tumor formation induced by polyoma middle T antigen (84). It will be interesting to see the impact of these mice on research into prostate cancer formation and progression induced by prostate-specific PTEN deficiency.

Acknowledgement: Damu Tang is supported by grants from Cancer Research Society, Canadian Cancer Society (grant #: 319412) and CIHR and by funds from Urological Cancer Center for Research and Innovation (UCCRI), Hamilton. Yan Gu is supported by Studentship provided by Ontario Graduate Scholarships and Research Institute of St Joe's Hamilton.

Conflicts of interest: The authors declare no potential conflict of interest with respect to research, authorship and/or publication of this chapter.

REFERENCES

1. Siegel R, Ward E, Brawley O, Jemal A. Cancer statistics, 2011: the impact of eliminating socioeconomic and racial disparities on premature cancer deaths. CA: a cancer journal for clinicians. 2011;61(4):212–36. https://doi.org/10.3322/caac.20121
2. Ross JS. The androgen receptor in prostate cancer: therapy target in search of an integrated diagnostic test. Adv Anat Pathol. 2007;14(5):353–7. https://doi.org/10.1097/PAP.0b013e31814a52c4
3. Moon C, Park JC, Chae YK, Yun JH, Kim S. Current status of experimental therapeutics for prostate cancer. Cancer Lett. 2008;266(2):116–34. https://doi.org/10.1016/j.canlet.2008.02.065
4. Bagnall P. Diagnosis and treatment of prostate cancer. Nurs Times. 2014;110(9):12–5.
5. Zaorsky NG, Raj GV, Trabulsi EJ, Lin J, Den RB. The dilemma of a rising prostate-specific antigen level after local therapy: what are our options? Semin Oncol. 2013;40(3):322–36. https://doi.org/10.1053/j.seminoncol.2013.04.011
6. Shipley WU, Seiferheld W, Lukka HR, Major PP, Heney NM, Grignon DJ, et al. Radiation with or without Antiandrogen Therapy in Recurrent Prostate Cancer. N Engl J Med. 2017;376(5):417–28. https://doi.org/10.1056/NEJMoa1607529
7. Semenas J, Allegrucci C, Boorjian SA, Mongan NP, Persson JL. Overcoming drug resistance and treating advanced prostate cancer. Curr Drug Targets. 2012;13(10):1308–23. https://doi.org/10.2174/138945012802429615
8. Ojo D, Lin X, Wong N, Gu Y, Tang D. Prostate Cancer Stem-like Cells Contribute to the Development of Castration-Resistant Prostate Cancer. Cancers. 2015;7(4):2290–308. https://doi.org/10.3390/cancers7040890
9. de Bono JS, Logothetis CJ, Molina A, Fizazi K, North S, Chu L, et al. Abiraterone and increased survival in metastatic prostate cancer. N Engl J Med. 2011;364(21):1995–2005. https://doi.org/10.1056/NEJMoa1014618
10. Scher HI, Fizazi K, Saad F, Taplin ME, Sternberg CN, Miller K, et al. Increased survival with enzalutamide in prostate cancer after chemotherapy. N Engl J Med. 2012;367(13):1187–97. https://doi.org/10.1056/NEJMoa1207506
11. Chaturvedi S, Garcia JA. Novel agents in the management of castration resistant prostate cancer. J Carcinog. 2014;13:5. https://doi.org/10.4103/1477-3163.128185
12. Drake CG. Prostate cancer as a model for tumour immunotherapy. Nat Rev Immunol. 2010;10(8):580–93. https://doi.org/10.1038/nri2817
13. Mei W, Gu Y, Jiang Y, Major P, Tang D. Circulating cell-free DNA is a potential prognostic biomarker of metastatic castration-resistant prostate cancer for taxane therapy. AME Med J. 2018;3(68):1–5. https://doi.org/10.21037/amj.2018.06.01
14. Buonaguro L, Petrizzo A, Tornesello ML, Buonaguro FM. Translating tumor antigens into cancer vaccines. Clin Vaccine Immunol. 2011;18(1):23–34. https://doi.org/10.1128/CVI.00286-10
15. de Paula Peres L, da Luz FA, Dos Anjos Pultz B, Brigido PC, de Araujo RA, Goulart LR, et al. Peptide vaccines in breast cancer: The immunological basis for clinical response. Biotechnol Adv. 2015;33(8):1868–77. https://doi.org/10.1016/j.biotechadv.2015.10.013
16. Correale P, Walmsley K, Nieroda C, Zaremba S, Zhu M, Schlom J, et al. In vitro generation of human cytotoxic T lymphocytes specific for peptides derived from prostate-specific antigen. J Natl Cancer Inst. 1997;89(4):293–300. https://doi.org/10.1093/jnci/89.4.293
17. Corman JM, Sercarz EE, Nanda NK. Recognition of prostate-specific antigenic peptide determinants by human CD4 and CD8 T cells. Clin Exp Immunol. 1998;114(2):166–72. https://doi.org/10.1046/j.1365-2249.1998.00678.x
18. Kufe DW. MUC1-C oncoprotein as a target in breast cancer: activation of signaling pathways and therapeutic approaches. Oncogene. 2013;32(9):1073–81. https://doi.org/10.1038/onc.2012.158
19. Kufe DW. Mucins in cancer: function, prognosis and therapy. Nature reviews Cancer. 2009;9(12):874–85. https://doi.org/10.1038/nrc2761
20. Ceriani RL, Thompson K, Peterson JA, Abraham S. Surface differentiation antigens of human mammary epithelial cells carried on the human milk fat globule. Proc Natl Acad Sci U S A. 1977;74(2):582–6. https://doi.org/10.1073/pnas.74.2.582

21. Gendler SJ. MUC1, the renaissance molecule. J Mammary Gland Biol Neoplasia. 2001;6(3):339–53. https://doi.org/10.1023/A:1011379725811
22. Ho SB, Niehans GA, Lyftogt C, Yan PS, Cherwitz DL, Gum ET, et al. Heterogeneity of mucin gene expression in normal and neoplastic tissues. Cancer Res. 1993;53(3):641–51.
23. Parmley RR, Gendler SJ. Cystic fibrosis mice lacking Muc1 have reduced amounts of intestinal mucus. J Clin Invest. 1998;102(10):1798–806. https://doi.org/10.1172/JCI3820
24. Ligtenberg MJ, Kruijshaar L, Buijs F, van Meijer M, Litvinov SV, Hilkens J. Cell-associated episialin is a complex containing two proteins derived from a common precursor. J Biol Chem. 1992;267(9): 6171–7. https://doi.org/10.1016/S0021-9258(18)42677-4
25. Levitin F, Stern O, Weiss M, Gil-Henn C, Ziv R, Prokocimer Z, et al. The MUC1 SEA module is a self-cleaving domain. J Biol Chem. 2005;280(39):33374–86. https://doi.org/10.1074/jbc.M506047200
26. Hanisch FG, Muller S. MUC1: the polymorphic appearance of a human mucin. Glycobiology. 2000;10(5):439–49. https://doi.org/10.1093/glycob/10.5.439
27. Gendler SJ, Lancaster CA, Taylor-Papadimitriou J, Duhig T, Peat N, Burchell J, et al. Molecular cloning and expression of human tumor-associated polymorphic epithelial mucin. J Biol Chem. 1990;265(25):15286–93. https://doi.org/10.1016/S0021-9258(18)77254-2
28. Nath S, Mukherjee P. MUC1: a multifaceted oncoprotein with a key role in cancer progression. Trends Mol Med. 2014;20(6):332–42. https://doi.org/10.1016/j.molmed.2014.02.007
29. Lau SK, Weiss LM, Chu PG. Differential expression of MUC1, MUC2, and MUC5AC in carcinomas of various sites: an immunohistochemical study. Am J Clin Pathol. 2004;122(1):61–9. https://doi.org/10.1309/9R6673QEC06D86Y4
30. Strawbridge RJ, Nister M, Brismar K, Li C, Lindstrom S. Influence of MUC1 genetic variation on prostate cancer risk and survival. Eur J Hum Genet. 2008;16(12):1521–5. https://doi.org/10.1038/ejhg.2008.131
31. Wong N, Major P, Kapoor A, Wei F, Yan J, Aziz T, et al. Amplification of MUC1 in prostate cancer metastasis and CRPC development. Oncotarget. 2016;7(50):83115–33. https://doi.org/10.18632/oncotarget.13073
32. Borno ST, Fischer A, Kerick M, Falth M, Laible M, Brase JC, et al. Genome-wide DNA methylation events in TMPRSS2-ERG fusion-negative prostate cancers implicate an EZH2-dependent mechanism with miR-26a hypermethylation. Cancer Discov. 2012;2(11):1024–35. https://doi.org/10.1158/2159-8290.CD-12-0041
33. Kumar-Sinha C, Tomlins SA, Chinnaiyan AM. Recurrent gene fusions in prostate cancer. Nat Rev Cancer. 2008;8(7):497–511. https://doi.org/10.1038/nrc2402
34. Zoni E, Karkampouna S, Thalmann GN, Kruithof-de Julio M, Spahn M. Emerging aspects of microRNA interaction with TMPRSS2-ERG and endocrine therapy. Mol Cell Endocrinol. 2018;462 (Pt A):9–16. https://doi.org/10.1016/j.mce.2017.02.009
35. Lapointe J, Li C, Higgins JP, van de Rijn M, Bair E, Montgomery K, et al. Gene expression profiling identifies clinically relevant subtypes of prostate cancer. Proc Natl Acad Sci U S A. 2004;101(3): 811–6. https://doi.org/10.1073/pnas.0304146101
36. Taylor BS, Schultz N, Hieronymus H, Gopalan A, Xiao Y, Carver BS, et al. Integrative genomic profiling of human prostate cancer. Cancer Cell. 2010;18(1):11–22. https://doi.org/10.1016/j.ccr.2010.05.026
37. Burchell J, Gendler S, Taylor-Papadimitriou J, Girling A, Lewis A, Millis R, et al. Development and characterization of breast cancer reactive monoclonal antibodies directed to the core protein of the human milk mucin. Cancer Res. 1987;47(20):5476–82.
38. Schut IC, Waterfall PM, Ross M, O'Sullivan C, Miller WR, Habib FK, et al. MUC1 expression, splice variant and short form transcription (MUC1/Z, MUC1/Y) in prostate cell lines and tissue. BJU Int. 2003;91(3):278–83. https://doi.org/10.1046/j.1464-410X.2003.03062.x
39. Burke PA, Gregg JP, Bakhtiar B, Beckett LA, Denardo GL, Albrecht H, et al. Characterization of MUC1 glycoprotein on prostate cancer for selection of targeting molecules. Int J Oncol. 2006;29(1):49–55. https://doi.org/10.3892/ijo.29.1.49
40. Cozzi PJ, Wang J, Delprado W, Perkins AC, Allen BJ, Russell PJ, et al. MUC1, MUC2, MUC4, MUC5AC and MUC6 expression in the progression of prostate cancer. Clin Exp Metastasis. 2005;22(7):565–73. https://doi.org/10.1007/s10585-005-5376-z

41. Zhang S, Zhang HS, Reuter VE, Slovin SF, Scher HI, Livingston PO. Expression of potential target antigens for immunotherapy on primary and metastatic prostate cancers. Clin Cancer Res. 1998;4(2):295–302.
42. Yamamoto M, Bhavanandan VP, Nakamori S, Irimura T. A novel monoclonal antibody specific for sialylated MUC1 mucin. Jpn J Cancer Res. 1996;87(5):488–96. https://doi.org/10.1111/j.1349-7006.1996.tb00250.x
43. Takeuchi H, Kato K, Denda-Nagai K, Hanisch FG, Clausen H, Irimura T. The epitope recognized by the unique anti-MUC1 monoclonal antibody MY.1E12 involves sialyl alpha 2–3galactosyl beta 1–3N-acetylgalactosaminide linked to a distinct threonine residue in the MUC1 tandem repeat. J Immunol Methods. 2002;270(2):199–209. https://doi.org/10.1016/S0022-1759(02)00298-3
44. Arai T, Fujita K, Fujime M, Irimura T. Expression of sialylated MUC1 in prostate cancer: relationship to clinical stage and prognosis. Int J Urol. 2005;12(7):654–61. https://doi.org/10.1111/j.1442-2042.2005.01112.x
45. Chen Z, Gulzar ZG, St Hill CA, Walcheck B, Brooks JD. Increased expression of GCNT1 is associated with altered O-glycosylation of PSA, PAP, and MUC1 in human prostate cancers. Prostate. 2014;74(10):1059–67. https://doi.org/10.1002/pros.22826
46. Rabiau N, Dechelotte P, Guy L, Satih S, Bosviel R, Fontana L, et al. Immunohistochemical staining of mucin 1 in prostate tissues. In Vivo. 2009;23(2):203–7.
47. O'Connor JC, Julian J, Lim SD, Carson DD. MUC1 expression in human prostate cancer cell lines and primary tumors. Prostate Cancer Prostatic Dis. 2005;8(1):36–44.
48. Rybak AP, He L, Kapoor A, Cutz JC, Tang D. Characterization of sphere-propagating cells with stem-like properties from DU145 prostate cancer cells. Biochimica et biophysica acta. 2011;1813(5):683–94. https://doi.org/10.1016/j.bbamcr.2011.01.018
49. Hikita ST, Kosik KS, Clegg DO, Bamdad C. MUC1* mediates the growth of human pluripotent stem cells. PloS one. 2008;3(10):e3312. https://doi.org/10.1371/journal.pone.0003312
50. Mei W, Lin X, Kapoor A, Gu Y, Zhao K, Tang D. The Contributions of Prostate Cancer Stem Cells in Prostate Cancer Initiation and Metastasis. Cancers. 2019;11(4). https://doi.org/10.3390/cancers11040434
51. Lin X, Gu Y, Kapoor A, Wei F, Aziz T, Ojo D, et al. Overexpression of MUC1 and Genomic Alterations in Its Network Associate with Prostate Cancer Progression. Neoplasia. 2017;19(11):857–67. https://doi.org/10.1016/j.neo.2017.06.006
52. Yasumizu Y, Rajabi H, Jin C, Hata T, Pitroda S, Long MD, et al. MUC1-C regulates lineage plasticity driving progression to neuroendocrine prostate cancer. Nat Commun. 2020;11(1):338. https://doi.org/10.1038/s41467-019-14219-6
53. Rajabi H, Ahmad R, Jin C, Joshi MD, Guha M, Alam M, et al. MUC1-C oncoprotein confers androgen-independent growth of human prostate cancer cells. Prostate. 2012;72(15):1659–68. https://doi.org/10.1002/pros.22519
54. Rajabi H, Joshi MD, Jin C, Ahmad R, Kufe D. Androgen receptor regulates expression of the MUC1-C oncoprotein in human prostate cancer cells. Prostate. 2011;71(12):1299–308. https://doi.org/10.1002/pros.21344
55. Mitchell S, Abel P, Ware M, Stamp G, Lalani E. Phenotypic and genotypic characterization of commonly used human prostatic cell lines. BJU Int. 2000;85(7):932–44. https://doi.org/10.1046/j.1464-410x.2000.00606.x
56. Evangelou A, Letarte M, Marks A, Brown TJ. Androgen modulation of adhesion and antiadhesion molecules in PC-3 prostate cancer cells expressing androgen receptor. Endocrinology. 2002;143(10):3897–904. https://doi.org/10.1210/en.2002-220156
57. Mitchell S, Abel P, Madaan S, Jeffs J, Chaudhary K, Stamp G, et al. Androgen-dependent regulation of human MUC1 mucin expression. Neoplasia. 2002;4(1):9–18. https://doi.org/10.1038/sj.neo.7900194
58. Huang DM, Guh JH, Chueh SC, Teng CM. Modulation of anti-adhesion molecule MUC-1 is associated with arctiin-induced growth inhibition in PC-3 cells. Prostate. 2004;59(3):260–7. https://doi.org/10.1002/pros.10364
59. Chachadi VB, Ali MF, Cheng PW. Prostatic cell-specific regulation of the synthesis of MUC1-associated sialyl Lewis a. PloS one. 2013;8(2):e57416. https://doi.org/10.1371/journal.pone.0057416

60. Hey NA, Aplin JD. Sialyl-Lewis x and Sialyl-Lewis a are associated with MUC1 in human endometrium. Glycoconj J. 1996;13(5):769–79. https://doi.org/10.1007/BF00702341
61. Zhang K, Baeckstrom D, Brevinge H, Hansson GC. Comparison of sialyl-Lewis a-carrying CD43 and MUC1 mucins secreted from a colon carcinoma cell line for E-selectin binding and inhibition of leukocyte adhesion. Tumour Biol. 1997;18(3):175–87. https://doi.org/10.1159/000218028
62. Fernandez-Rodriguez J, Dwir O, Alon R, Hansson GC. Tumor cell MUC1 and CD43 are glycosylated differently with sialyl-Lewis a and x epitopes and show variable interactions with E-selectin under physiological flow conditions. Glycoconj J. 2001;18(11–12):925–30. https://doi.org/10.1023/A:1022208727512
63. Borsig L. Selectins in cancer immunity. Glycobiology. 2018;28(9):648–55. https://doi.org/10.1093/glycob/cwx105
64. Xiang S, Zhang Q, Tang Q, Zheng F, Wu J, Yang L, et al. Activation of AMPKalpha mediates additive effects of solamargine and metformin on suppressing MUC1 expression in castration-resistant prostate cancer cells. Sci Rep. 2016;6:36721. https://doi.org/10.1038/srep36721
65. Papadopoulos I, Sivridis E, Giatromanolaki A, Koukourakis MI. Tumor angiogenesis is associated with MUC1 overexpression and loss of prostate-specific antigen expression in prostate cancer. Clin Cancer Res. 2001;7(6):1533–8.
66. Okamoto T, Yoneyama MS, Hatakeyama S, Mori K, Yamamoto H, Koie T, et al. Core2 O-glycan-expressing prostate cancer cells are resistant to NK cell immunity. Mol Med Rep. 2013;7(2):359–64. https://doi.org/10.3892/mmr.2012.1189
67. Liang X, Li Z, Men Q, Li Y, Li H, Chong T. miR-326 functions as a tumor suppressor in human prostatic carcinoma by targeting Mucin1. Biomed Pharmacother. 2018;108:574–83. https://doi.org/10.1016/j.biopha.2018.09.053
68. Genitsch V, Zlobec I, Thalmann GN, Fleischmann A. MUC1 is upregulated in advanced prostate cancer and is an independent prognostic factor. Prostate Cancer Prostatic Dis. 2016;19(3):242–7. https://doi.org/10.1038/pcan.2016.11
69. Eminaga O, Wei W, Hawley SJ, Auman H, Newcomb LF, Simko J, et al. MUC1 Expression by Immunohistochemistry Is Associated with Adverse Pathologic Features in Prostate Cancer: A Multi-Institutional Study. PloS one. 2016;11(11):e0165236. https://doi.org/10.1371/journal.pone.0165236
70. Severi G, FitzGerald LM, Muller DC, Pedersen J, Longano A, Southey MC, et al. A three-protein biomarker panel assessed in diagnostic tissue predicts death from prostate cancer for men with localized disease. Cancer Med. 2014;3(5):1266–74. https://doi.org/10.1002/cam4.281
71. Jiang Y, Mei W, Gu Y, Lin X, He L, Zeng H, et al. Construction of a set of novel and robust gene expression signatures predicting prostate cancer recurrence. Mol Oncol. 2018;12(9):1559–78. https://doi.org/10.1002/1878-0261.12359
72. Andren O, Fall K, Andersson SO, Rubin MA, Bismar TA, Karlsson M, et al. MUC-1 gene is associated with prostate cancer death: a 20-year follow-up of a population-based study in Sweden. Br J Cancer. 2007;97(6):730–4. https://doi.org/10.1038/sj.bjc.6603944
73. Premaratne P, Welen K, Damber JE, Hansson GC, Backstrom M. O-glycosylation of MUC1 mucin in prostate cancer and the effects of its expression on tumor growth in a prostate cancer xenograft model. Tumour Biol. 2011;32(1):203–13. https://doi.org/10.1007/s13277-010-0114-9
74. Sanchez C, Chan R, Bajgain P, Rambally S, Palapattu G, Mims M, et al. Combining T-cell immunotherapy and anti-androgen therapy for prostate cancer. Prostate Cancer Prostatic Dis. 2013;16(2):123–31, S1. https://doi.org/10.1038/pcan.2012.49
75. Gao T, Cen Q, Lei H. A review on development of MUC1-based cancer vaccine. Biomed Pharmacother. 2020;132:110888. https://doi.org/10.1016/j.biopha.2020.110888
76. Palmer M, Parker J, Modi S, Butts C, Smylie M, Meikle A, et al. Phase I study of the BLP25 (MUC1 peptide) liposomal vaccine for active specific immunotherapy in stage IIIB/IV non-small-cell lung cancer. Clin Lung Cancer. 2001;3(1):49–57; https://doi.org/10.3816/CLC.2001.n.018
77. North SA, Graham K, Bodnar D, Venner P. A pilot study of the liposomal MUC1 vaccine BLP25 in prostate specific antigen failures after radical prostatectomy. J Urol. 2006;176(1):91–5. https://doi.org/10.1016/S0022-5347(06)00494-0

78. Scheid E, Major P, Bergeron A, Finn OJ, Salter RD, Eady R, et al. Tn-MUC1 DC Vaccination of Rhesus Macaques and a Phase I/II Trial in Patients with Nonmetastatic Castrate-Resistant Prostate Cancer. Cancer Immunol Res. 2016;4(10):881–92. https://doi.org/10.1158/2326-6066.CIR-15-0189
79. Westdorp H, Creemers JHA, van Oort IM, Schreibelt G, Gorris MAJ, Mehra N, et al. Blood-derived dendritic cell vaccinations induce immune responses that correlate with clinical outcome in patients with chemo-naive castration-resistant prostate cancer. J Immunother Cancer. 2019;7(1):302. https://doi.org/10.1186/s40425-019-0787-6
80. Dreicer R, Stadler WM, Ahmann FR, Whiteside T, Bizouarne N, Acres B, et al. MVA-MUC1-IL2 vaccine immunotherapy (TG4010) improves PSA doubling time in patients with prostate cancer with biochemical failure. Invest New Drugs. 2009;27(4):379–86. https://doi.org/10.1007/s10637-008-9187-3
81. Joshi MD, Ahmad R, Yin L, Raina D, Rajabi H, Bubley G, et al. MUC1 oncoprotein is a druggable target in human prostate cancer cells. Mol Cancer Ther. 2009;8(11):3056–65. https://doi.org/10.1158/1535-7163.MCT-09-0646
82. Mazor Y, Noy R, Wels WS, Benhar I. chFRP5-ZZ-PE38, a large IgG-toxin immunoconjugate outperforms the corresponding smaller FRP5(Fv)-ETA immunotoxin in eradicating ErbB2-expressing tumor xenografts. Cancer Lett. 2007;257(1):124–35. https://doi.org/10.1016/j.canlet.2007.07.009
83. Pichinuk E, Chalik M, Benhar I, Ginat-Koton R, Ziv R, Smorodinsky NI, et al. In vivo anti-MUC1(+) tumor activity and sequences of high-affinity anti-MUC1-SEA antibodies. Cancer Immunol Immunother: CII. 2020;69(7):1337–52. https://doi.org/10.1007/s00262-020-02547-2
84. Spicer AP, Rowse GJ, Lidner TK, Gendler SJ. Delayed mammary tumor progression in Muc-1 null mice. J Biol Chem. 1995;270(50):30093–101. https://doi.org/10.1074/jbc.270.50.30093
85. Peat N, Gendler SJ, Lalani N, Duhig T, Taylor-Papadimitriou J. Tissue-specific expression of a human polymorphic epithelial mucin (MUC1) in transgenic mice. Cancer Res. 1992;52(7):1954–60.

10

The Usefulness of STEAP Proteins in Prostate Cancer Clinical Practice

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Doi: https://doi.org/10.36255/exonpublications.prostatecancer.steap.2021

Abstract: Prostate cancer is a multifactorial disease and the second most common cancer diagnosed in men worldwide. The six transmembrane epithelial antigen of prostate (STEAP) proteins seem to be involved in prostate tumorigenesis. The STEAP proteins are differentially expressed in prostate cancer cells, and survival analysis reveal that prostate cancer patients with high levels of STEAP1 have poor survival outcomes. In contrast, high expression of STEAP4 offers a better prognosis. This chapter provides an overview of the role of STEAP proteins in prostate cancer. The structure, biological functions, and the potential prognostic significance of each of the four members of the STEAP family in prostate cancer are discussed.

Keywords: biomarker; prognosis; prostate cancer; STEAP proteins; survival

In: *Prostate Cancer*. Bott SRJ, Ng KL (Editors). Exon Publications, Brisbane, Australia. ISBN: 978-0-6450017-5-4; Doi: https://doi.org/10.36255/exonpublications.prostatecancer.2021

INTRODUCTION

Prostate cancer is the second most diagnosed cancer and the sixth leading cause of cancer-related death in men in the Western world. Each year, 1.6 million men are diagnosed with this neoplasia, and 366,000 men die of prostate cancer (1). In 2040, 2.3 million new cases and 740, 000 prostate cancer-related deaths are estimated to occur worldwide due to the growth and aging of population (1). Endogenous (age, family history, ethnicity, hormones, and oxidative stress) and exogenous (dietary factors, physical inactivity, obesity, environmental factors, occupation, smoking) risk factors contribute to the risk of developing prostate cancer. However, older age, black race, and a family history of the disease are the best-established risk factors for prostate cancer (2).

The main biomarker used in clinical practice for prostate cancer screening is the serum level of prostate-specific antigen (PSA). However, several factors may affect PSA levels resulting in a considerable number of false-positives (3). The low specificity of PSA in the diagnosis of prostate cancer is a clinical problem. There is an urgent need to identify new biomarkers for early detection of the disease, and to improve patients' stratification and better define targeted therapies and clinical management of prostate cancer.

The human six-transmembrane epithelial antigen of prostate (STEAP) family of proteins comprises four members, namely STEAP1 to STEAP4, which are involved in numerous biological processes including the control of cell proliferation and apoptosis (4), oxidative stress (5) and molecular trafficking in exocytic and endocytic pathways (6). Cumulative evidence has pointed out STEAP family members as putative biomarkers, as well as therapeutic targets, in several types of human cancers, particularly in prostate cancer (7–11). However, the clinical significance of the expression of STEAP proteins for prostate cancer development is still scarce, and further analysis is required to ascertain their usefulness as prognostic biomarkers. This chapter first provides an overview of the structure and biological functions of STEAP proteins, followed by a discussion on their role in prostate cancer. Their putative role in tumorigenesis and prognosis of prostate cancer, based on datasets retrieved from the cBioPortal (12) and CANCERTOOL (13) public databases are presented.

STRUCTURE AND ROLE OF STEAP PROTEINS

All STEAPs have a characteristic six-transmembrane helix with intracellular N- and C-terminal domains, with a homologous architecture to ion-channels and/or transporter proteins (6, 8, 14, 15). The C-terminal domain is similar to the transmembrane domain (TMD) of the yeast ferric reductase (FRE) family of b-type cytochrome metalloreductases, whereas the N-terminal is comparable to the archaeal and bacterial F_{420}:NADPH-oxidoreductase (FNO)-binding proteins and to human NADPH-oxidoreductase domains (OxRD) (6, 8, 14, 15). The FNO-like domain reinforces the importance of STEAPs in the uptake and reduction of molecular oxygen and chelation of metal ions Fe^{3+} and Cu^{2+}, and the involvement of these proteins in transmembrane-electron transport through the intracellular binding of NAD and FMN nucleotides to a conserved single heme-binding

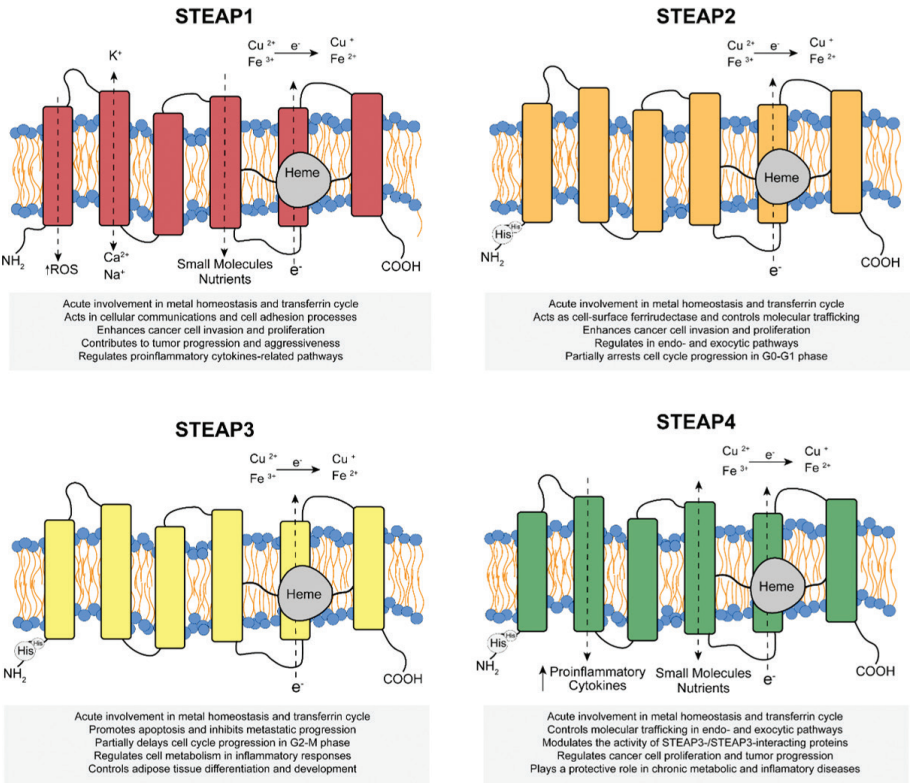

Figure 1. Schematic structure of STEAP family members. Representation of STEAP1, STEAP2, STEAP3 and STEAP4 proteins and their main cellular and biological functions.

histidine residue and a flavin-binding site (6, 14, 15). Also, STEAPs share a YXXØ consensus sequence responsible for targeting transmembrane proteins to lysosomes and endosomes, and the Rossmannfold (GxGxxG/A motif) that binds NAD and FMN coenzymes (6). Besides maintaining cellular metal homeostasis, STEAPs are implicated in several biological processes, such as oxidative stress response, inflammation, metabolism, invasion, proliferation, growth, and apoptosis (Figure 1) (8).

STEAP1

The *STEAP1* gene is located on chromosome 7q21.13 close to *STEAP1B*, *STEAP2*, and *STEAP4*, in a region that contains a cluster of genes predicted to encode transmembrane proteins. It encodes an mRNA of 1.3 kb that is translated into a mature protein of 339 amino acids (UniProt ID: Q9UHE8, 39.851 kDa). The STEAP1 protein is composed of six-transmembrane domains with cytosolic C- and N-terminals connected by three extra- and two intracellular loops preferentially

located at tight and gap junctions (16). Unlike other STEAPs, STEAP1 lacks the N-terminal NADPH-oxidoreductase, the FNO-like domain, and the Rossmann fold (8, 14). Nevertheless, STEAP1 partially co-localizes with transferrin (Tf), transferrin receptor (TfR), and lysosomes or endosomes, suggesting an involvement in metal homeostasis (6, 17). A recent study indicated that dithionite-reduced purified STEAP1 is capable of reducing metal-ion complexes and molecular oxygen through a conserved heme-binding site (18). Furthermore, the amino acid sequence, the transmembrane topology, and cellular membrane localization of STEAP1 indicate that it may act as an ion channel or transporter, modulating the concentration of small molecules, ions and nutrients, and also releasing soluble cytokines and chemokines (19). These features pointed out the role of STEAP1 in cellular communication and in cell adhesion processes (19, 20). However, the recent cryogenic electron microscopy (cryo-EM) structure of STEAP1 transmembrane domain bound to an antigen-binding fragment of an antibody (mAb 120.545) revealed a trimeric arrangement quite distinct from typical ion channels or transporters (21). The proposed unit indicated that STEAP1 is a functional reductase in heterodimers complexes with other STEAP paralogs with a domain-swapped architecture with the intracellular OxRD positioned beneath the TMD of the adjacent promoter (21, 22). This arrangement supports a model in which the heme-binding site recruit and orient intracellular electron-donating substrates bound to an adjacent STEAP2–4 subunit, enabling transmembrane electron transport and the reduction of extracellular metal-ion complexes (18, 21). These structural features highlight the usefulness of STEAP1 as a promising therapeutic target and biomarker for cancer and encouraged the development of strategies targeting STEAP1. *In vitro* and *in vivo* studies revealed mobilized dendritic cells and immunogenic STEAP1-derived peptides suitable for recognition by cytotoxic T lymphocytes for further development of anti-cancer vaccines (23). Humanized variant of anti-STEAP1 monoclonal antibody (mAb 120.545) is currently used in prostate cancer clinical trials as an antibody-drug conjugate (DSTP3086S) (24), and as a radiolabeled antibody (89Zr-DFO-MSTP2109A) for PET imaging (24, 25). In addition, several studies exploring the role of STEAP1 in cancer cells showed that its overexpression inhibits apoptosis, enhances cell proliferation and invasion, and induces epithelial to mesenchymal transition, ultimately contributing to tumor progression and aggressiveness (4, 5, 26–30).

STEAP2

The *STEAP2* gene is located on chromosome 7q21.13, close to *STEAP1* and *STEAP4*, and encodes an mRNA of 2.2 kb, which generates a protein with 490 amino acids (UniProt ID: Q8NFT2, 52.052 kDa). The protein shuttles between the plasma membrane and Golgi complex in prostate epithelial cells (31). Its association with the trans-Golgi network and early endosomes suggests the involvement of STEAP2 in endo- and exocytic pathways, acting as a regulator of processing, secreting and sorting mechanisms of prostate-specific proteins, or as a receptor for endo- or exogenous ligands (31, 32). Moreover, as STEAP2 co-localizes with Tf and TfR, the protein contributes to iron and copper reduction, and plays a role in the endosomal Tf cycle of erythroid cells, then controlling the

molecular trafficking and availability of metals (6, 17). STEAP2 regulates several genes involved in cell cycle progression. Downregulation of STEAP2 results in a partial cell cycle arrest in the G0/G1 phase, suppression of prostate cancer cell proliferation, invasiveness, and metastatic potential (33). In contrast, STEAP2 overexpression increases the migration and invasion abilities of prostate cancer cells, and is associated with advanced cancer stage and histologic grading (34, 35). Although STEAP2 presents proliferative features and acts as a pro-survival factor, more studies exploring the molecular and signaling pathways underlying prostate cancer are required.

STEAP3

The *STEAP3* gene is located on chromosome 2q14.2 and encodes an mRNA of 4.3 kb, generating a protein with 488 amino acids (UniProt ID: Q658P3, 54.601 kDa). The predicted structure of STEAP3 indicates six-transmembrane domains at C-terminal region, a cytosolic OxRD at the N-terminal counterpart and two conserved histidine residues (36). The crystal structure of cytoplasmic OxRD showed similarity with integral membrane cytochromes (37). The STEAP3 has unique structural and biochemical properties, revealing an FNO-like domain with a dimer interface and substrate binding sites, to direct electron transfer from the cytosol to a single b-type heme moiety predicted to be fixed within the TMD (14, 37). Furthermore, the N-terminal OxRD was found to dimerize, suggesting that STEAP3 is active as a homo- and/or heterodimer, and is found in cellular membranes to permit intercellular electron flow (14, 37). STEAP3 is mainly located in the plasma and endosomal membranes, and as a component of trans-Golgi network and endosomal-vesicular compartments (38). Also, STEAP3 is essential for the assembly of exosomes and vesicular proteins trafficking (39), and extracellular matrix organization (40). Moreover, the partial co-localization with Tf, TfR and divalent metal transporter 1 (DMT1), along with STEAP3 structural arrangement, suggest an important biological role of this protein in ferric compounds metabolism and erythroid transferrin cycle (14,41). These functions were confirmed through the analysis of STEAP3 crystal structure, which postulated that the protein might function within a large complex with DMT1 and Tf:TfR, ensuring the reduction and uptake of iron and copper at the cell surface (37). Several studies pointed out that STEAP3 is the major ferric reductase and deeply involved in regulating metal homeostasis in developing erythrocytes, macrophages, hepatocytes and endosomes (41). Therefore, tight regulation of intracellular iron is crucial to control cancer cell proliferation and apoptosis, and inhibiting the metastatic process (42). Furthermore, STEAP3 may be considered a tumor suppressor gene, acting as an intrinsic apoptosis factor. Studies showed that this protein suppresses the growth of human prostate cancer cells and could directly induce apoptosis through a caspase-3 dependent pathway (43, 44). On the other hand, the increased expression of STEAP3 is related to the progression of prostate cancer in late stages of disease, ultimately contributing to metastization (45). Altogether, available data highlight the importance of STEAP3 as a potential tumor suppressor protein, a feature that contrasts with other STEAPs.

STEAP4

The *STEAP4* gene is located on chromosome 7q21.12 and encodes an mRNA of 4.5 kb, and a protein composed of 459 amino acids (UniProt ID: Q687X5, 51.981 kDa). STEAP4 is located at the plasma membrane, near the nuclear region, where it co-localizes with the Golgi complex, the trans-Golgi network and early endosomes (46). As with STEAP2 and STEAP3, the STEAP4 protein is involved in molecular trafficking either in endo- or exocytic pathways, and might be critical to cellular and systemic metal homeostasis (6, 17). The STEAP4 protein is also found dispersed in the cytoplasm within vesicular-tubular structures or reticular shapes associated with the estrogen receptor (ER), wherein it acquires its active conformation (46). Initially, it was predicted that STEAP4 was an integral six-transmembrane metalloreductase composed of cytoplasmic N-terminal OxRD and a C-terminal TMD with six-membrane spanning α-helices enveloping a single heme-binding site (14). However, the recently solved cryo-EM structure of human STEAP4 demonstrated an aligned inter-subunit trimeric NADPH-FAD-heme domain-swapped assembly containing both OxRD and six-helical TMD (15, 22). This arrangement facilitates the transport of intracellular electrons from NADPH through membrane-embedded FAD that flips to anchor itself in the inner-membrane region of the adjacent STEAP subunit, and heme co-factors to chelated metal-ion complexes at the extracellular membrane side (14, 15, 22). Altogether, these biochemical and structural studies suggest that STEAP4 is a functional protein by establishing homo- or heterodimers with other STEAPs paralogs, which indicate that increased expression of STEAP4 could modulate the activity of STEAP3, and STEAP3-interacting proteins, such as NIX, MYT1, NIP3L, FAK-1, S100B, RHBDL4/RHBDD1 (14, 37, 47). Moreover, these interactions suggest a link to metal homeostasis, apoptosis, differentiation, and cell cycle progression (47). The STEAP4 protein is involved in cellular responses to nutritional and inflammatory signals, particularly as glucose homeostasis regulator in adipocytes, besides being widely associated with cardiac malfunctions, hepatic and mitochondrial metabolic dysfunctions, skeletal system-related disorders, and also as a suppressor of pro-inflammatory cytokines (47). In addition to its role in cellular or systemic homeostasis, STEAP4 is associated with tumorigenesis. Considering its metal reductase activity, an overexpression of STEAP4 increases oxidative stress, contributing to increased mutational rates, proliferation and progression of prostate cancer cells (48). The effects of STEAP4 on prostate cancer growth and survival are due to the modulation and regulation of the expression and activity of focal adhesion kinase (FAK) and activating transcription factor (ATF4) through increased intracellular reactive oxygen species, which depend on the OxRD of STEAP4 (48, 49). It was recently demonstrated that ATF4-target genes promote prostate cancer cell survival and are upregulated in late stages of the disease (50). These considerations indicate a protective role of STEAP4 in inflammatory stress in chronic metabolic and inflammatory diseases. Besides, they pointed out an active role of STEAP4 in cancer cell proliferation and tumor progression, highlighting the importance of understanding its putative function in disease-related environment.

STEAP IN PROSTATE CANCER

STEAP family members have been implicated in several human cancers, including prostate cancer. In non-neoplastic prostate, the expression of STEAP1 mRNA and protein are higher when compared to the other three family members (8). With the development of prostate cancer, the overall expression of STEAPs is dysregulated or reversed. STEAP1 expression is highly increased in prostate cancer in comparison with non-malignant tissues (Table 1) (16, 27, 28). Also, STEAP1 staining intensity correlated with tumor grading, suggesting that it is associated with malignant transformation and tumor aggressiveness (27, 28). The aggressiveness and prognosis of prostate cancer is traditionally determined by Gleason score, and a recent study by Burnell *et al.* showed that STEAP1 staining intensity in non-malignant tissue was weak, increasing slightly in Gleason 6 prostate cancer samples, and strongly from Gleason 7 onwards (7). Nowadays, public databases are widely used to corroborate basic research. The Prostate Adenocarcinoma dataset (51) from cBio Cancer Genomics Portal (https://cbioportal.org [accessed on December 2020]), was analyzed for STEAPs mRNA expression with a z-score threshold of ±1.8. Of the 150 patients queried, STEAP1 was overexpressed in 17%, and underexpressed in 0.8% of patients. Bioinformatics analysis using the CANCERTOOL software (http://genomics.cicbiogune.es/ [accessed on December 2020]), which uses transcriptomics databases for the most prevalent types of cancers, demonstrated that increased expression of STEAP1 correlates with the onset of prostate cancer and development of metastatic disease (Figure 2).

TABLE 1 **Relative expression levels of STEAP family members in human prostate cancer compared with non-malignant tissue**

		Expression pattern	Reference
STEAP1	mRNA	Increased	(16,27,28)
	Protein		
STEAP2	mRNA	Increased	(31,32)
	Protein		
STEAP3	mRNA	Decreased	(45)
	Protein		
STEAP4	mRNA	Increased	(46)
	Protein		

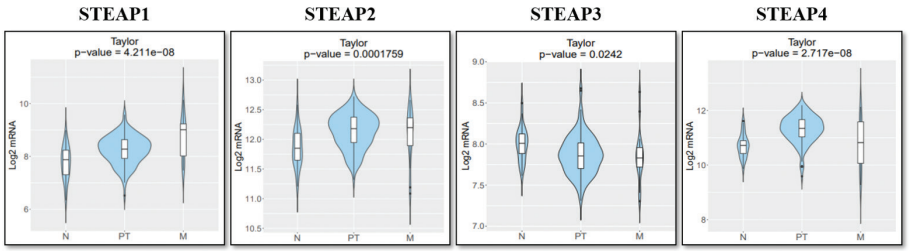

Figure 2. STEAPs expression with the progression of human prostate cancer using CANCERTOOL software. Violin plots show the expression of STEAPs in non-tumoral tissue (N), primary tumours (PT) and metastatic prostate cancer (M) specimens for Taylor dataset (51). This dataset has a cohort of 179 patients and is the only one with statistical significance for all STEAPs. However, the same results are verified in other dataset: for STEAP1, Grasso dataset (52); for STEAP2, Grasso (52), Tomlins (53) and Varambally (54) datasets; for STEAP4, Grasso (52) and Lapointe (55) dataset. Mean gene expression between the three groups was compared with an ANOVA test.

Regarding STEAP2, its expression levels were significantly higher in prostate carcinoma (Table 1). Several other studies have demonstrated the overexpression of STEAP2 in prostate cancer (31, 34, 35). Burnell *et al.* showed that STEAP2 overexpression is positively correlated with the Gleason score (7). From the cBioPortal, the Prostate Adenocarcinoma dataset (51) showed that STEAP2 overexpression occurred in 16% of cases, while underexpressed in 1.6% of the cases. CANCERTOOL analysis also indicated a significantly higher expression of STEAP2 considering the Taylor dataset (Figure 2).

In contrast to other STEAPs, Porkka *et al.* described that STEAP3 expression decreases with the onset of prostate malignancy, suggesting that this gene may acts as a prostate tumor suppressor (Table 1) (46). Public datasets are in agreement with this study. Analysis of the Prostate Adenocarcinoma dataset (51) showed a downregulation of STEAP3 in 18% of samples, whereas only 4% showed an overexpression. Also, CANCERTOOL software revealed a significantly diminished expression of STEAP3 in primary tumors and metastatic prostate cancer (Figure 2). However, Burnell *et al.* showed that there was no correlation between STEAP3 staining intensity and Gleason score (7). On the other hand, CANCERTOOL analysis indicated downregulation of STEAP3, and the low levels of STEAP3 are statistically correlated with high Gleason score.

STEAP4 subcellular localization is similar to that of STEAP2, and its expression also increases during prostate cancer development compared with non-malignant prostate tissue (Table 1) (46). Analysis of the Prostate Adenocarcinoma dataset (51) retrieved from cBioPortal showed an increased expression of STEAP4 in 37.3% of samples, and a decreased expression in 4.7% the of the samples. Using the CANCERTOOL software, it was demonstrated that the increased STEAP4 expression correlated with prostate malignancy and the development of metastatic prostate cancer (Figure 2). Similar to STEAP3, no correlation between STEAP4 overexpression and Gleason score was observed (7), however, CANCERTOOL analysis showed that overexpression of STEAP4 is positively associated with Gleason grade.

PROGNOSTIC VALUE OF STEAP PROTEINS IN PROSTATE CARCINOMA

STEAP family members are relatively new proteins and their potential as prognostic biomarkers have been demonstrated in breast cancer (56), glioblastoma (57, 58), Ewing tumors (30), skin disorders (59) and prostate cancer (7). A recent study that explored the use of STEAP proteins as possible prognostic indicators in prostate cancer showed that only STEAP4 was overexpressed, and significantly associated with relapse (7). To date, there are no studies evaluating the combined expression of different STEAP proteins. Thus, our intention in this chapter is to explore the association of STEAPs with prognosis of prostate cancer. A possible linear association between two variables, in this case, two genes, is analyzed by correlation coefficient (60), where -1 and 1 indicate a negative and positive perfect linear relationship, respectively. Using primary prostate tumor tissue dataset from online MERAV database (Metabolic gEne Rapid Visualizer, http://merav.wi.mit.edu/ [accessed on December 2020]) a strong positive correlation was found between STEAP1 and STEAP2 expression (Table 2). This observation is in accordance with data of Grunewald *et al.*, who noted that STEAP1 and STEAP2 seem to be significantly co-overexpressed across 59 cancer cell line entities (9), which suggests that STEAP2 is a likely candidate for heterodimerization with STEAP1. However, it is unknown if the combined expression of these two genes correlates with the overall survival of prostate cancer patients.

Using the same dataset retrieved from the cBioPortal (51), it was found that only STEAP1 overexpression is associated with the overall survival of prostate cancer patients (Figure 3A and Table 3). High expression of STEAP1 is associated with a shorter survival time, indicating a poor outcome. Concerning STEAP2, STEAP3 and STEAP4, no significant relationship was found between higher or low expression levels and the survival of prostate cancer patients (Table 3). These findings contradict the study of Burnell *et al.* (7) that reported a statistically significant difference between STEAP4 overexpression and overall survival, indicating that patients with higher STEAP4 levels were more likely to relapse earlier than those with medium or low expression levels (7). The source of the data, the number of patients per group, and the stratification of STEAPs expression may have caused the differences observed.

TABLE 2 **Gene correlation between of the STEAP family members**

	STEAP1	STEAP2	STEAP3	STEAP4
STEAP1	1	**0.9**	–0.09	0.43
STEAP2	**0.9**	1	–0.13	0.43
STEAP3	–0.09	–0.13	1	–0.03
STEAP4	0.43	0.43	–0.03	1

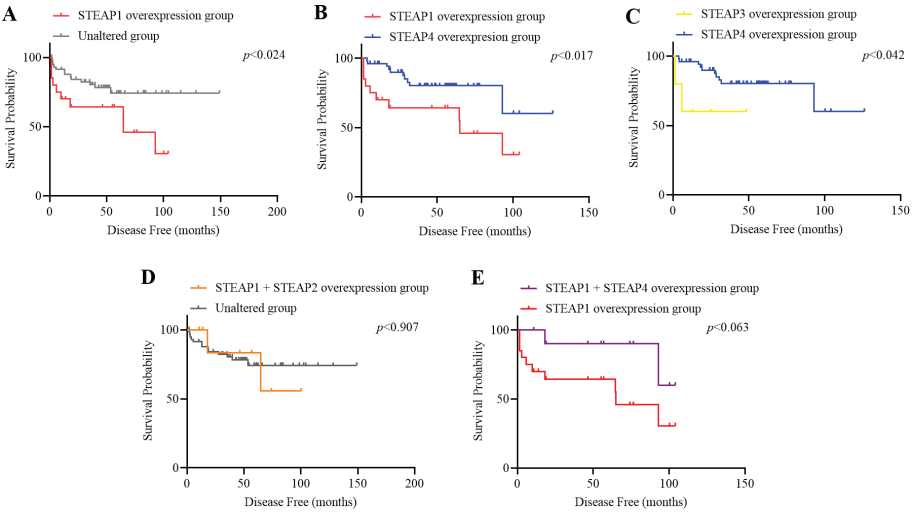

Figure 3. Expression of STEAP proteins and overall survival in prostate cancer patients. The Prostate Adenocarcinoma dataset (51), retrieved from cBioPoral, was stratified into groups with unaltered or overexpression of STEAPs, and different combinations were analyzed for overall survival. **A.** STEAP1 overexpression (n = 20) versus unaltered expression (n = 58) ($p < 0.024$). **B.** STEAP1 overexpression (n =20) versus STEAP4 overexpression (n = 52) ($p < 0.017$). **C.** STEAP3 overexpression (n = 5) versus STEAP4 overexpression (n = 52) ($p < 0.042$). **D.** STEAP1 overexpression plus STEAP2 overexpression (n = 8) versus unaltered expression (n = 58) ($p < 0.907$). **E.** STEAP1 overexpression plus STEAP4 overexpression (n = 11) versus STEAP1 overexpression (n = 20) ($p < 0.063$). Survival curves plotting fractional survival as a function of time was obtained using GraphPad Prisma 8.0.1.

As shown in Table 3 and considering all the possible associations between different STEAP proteins, patients with STEAP1 overexpression displayed significantly lower overall survival compared with patients with overexpression of STEAP4 (Figure 3B). Furthermore, patients with STEAP3 overexpression presented poor survival outcome when compared with patients overexpressing STEAP4 (Figure 3C). These two associations with statistical significance suggest that the overexpression of STEAP4 can be a predictor for patients with prostate cancer since they presented a better survival rate when compared with those overexpressing STEAP1 and/or STEAP3. Additionally, a strong trend for better survival was observed in prostate cancer patients that presented both high expression of STEAP1 and STEAP4 compared with the ones that only overexpress STEAP1 (Figure 3E). The overexpression of both STEAP1 and STEAP2 could mean a potential good prediction for prostate cancer patients (Figure 3D), since the combined expression of high levels of STEAP1 and high levels of STEAP2 presented a better survival rate when compared with samples overexpressing STEAP1 only (Figure 3A). Despite the study limitations, such as the low number of patients in some experimental groups, these findings provide a comprehensive analysis of the STEAP expression in prostate cancer and their application for predicting prognosis of prostate cancer.

TABLE 3 **Expression of STEAP family members correlated with overall survival of prostate cancer patients**

STEAP1 overexpression (n = 20) ***versus***		Unaltered expression (n = 58)	**$p < 0.024$**
		STEAP2 overexpression (n = 21)	$p < 0.108$
		STEAP3 underexpression (n = 24)	$p < 0.228$
		STEAP4 overexpression (n = 52)	**$p < 0.017$**
STEAP1 overexpression + **STEAP2 overexpression (n = 8)** ***versus***		Unaltered expression (n = 58)	$p < 0.907$
		STEAP1 overexpression (n = 20)	$p < 0.262$
		STEAP2 overexpression (n = 21)	$p < 0.938$
STEAP1 overexpression + **STEAP3 underexpression (n = 6)** ***versus***		Unaltered expression (n = 58)	$p < 0.789$
		STEAP1 overexpression (n = 20)	$p < 0.252$
		STEAP3 underexpression (n = 24)	$p < 0.687$
STEAP1 overexpression + **STEAP4 overexpression (n = 11)** ***versus***		Unaltered expression (n = 58)	$p < 0.640$
		STEAP1 overexpression (n = 20)	$p < 0.063$
		STEAP4 overexpression (n = 52)	$p < 0.639$
STEAP2 overexpression (n = 21) ***versus***		Unaltered expression (n = 58)	$p < 0.962$
		STEAP3 underexpression (n = 24)	$p < 0.549$
		STEAP4 overexpression (n = 52)	$p < 0.784$
STEAP2 overexpression + **STEAP3 underexpression (n = 7)** ***versus***		Unaltered expression (n = 58)	$p < 0.215$
		STEAP2 overexpression (n = 21)	$p < 0.307$
		STEAP3 underexpression (n = 24)	$p < 0.166$
STEAP2 overexpression + **STEAP4 overexpression (n = 13)** ***versus***		Unaltered expression (n = 58)	$p < 0.826$
		STEAP2 overexpression (n = 21)	$p < 0.784$
		STEAP4 overexpression (n = 52)	$p < 0.638$
STEAP3	underexpression (n = 24) ***versus***	Unaltered expression (n = 58)	$p < 0.696$
		STEAP4 overexpression (n = 52)	$p < 0.451$
	overexpression (n = 5) ***versus***	Unaltered expression (n = 58)	$p < 0.118$
		STEAP1 overexpression (n = 20)	$p < 0.795$
		STEAP2 overexpression (n = 21)	$p < 0.090$
		STEAP4 overexpression (n = 52)	**$p < 0.042$**
STEAP3 underexpression + **STEAP4 overexpression (n = 14)** ***versus***		Unaltered expression (n = 58)	$p < 0.566$
		STEAP3 underexpression (n = 24)	$p < 0.811$
		STEAP4 overexpression (n = 52)	$p< 0.399$
STEAP4	overexpression (n = 52) ***versus***	Unaltered expression (n = 58)	$p < 0.753$
	underexpression (n = 5) ***versus***	Unaltered expression (n = 58)	$p < 0.278$
		STEAP1 overexpression (n = 20)	$p < 0.829$
		STEAP2 overexpression (n = 21)	$p < 0.330$
		STEAP3 overexpression (n = 5)	$p < 0.821$
		STEAP3 underexpression (n = 24)	$p < 0.399$

Data was extracted from Prostate Adenocarcinoma (MSKCC, 2010) (51) of cBioPortal and statistically analyzed using the GraphPad Prisma 8.0.1 software. Statistically significant differences considered for *p*-values < 0.05 are highlighted in bold.

CONCLUSION

Analyzing available scientific literature and multiple public databases show that STEAP1, STEAP2, and STEAP4 are overexpressed in prostate tumors. In contrast, STEAP3 is underexpressed. The differential expressions of these proteins appear to be of prognostic value. Overexpression of STEAP1 overexpression is associated with poor clinical outcomes, whereas STEAP4 offers better overall survival and progression-free survival. However, further investigations in large scale clinical cohorts are needed to definitively confirm the prognostic value of the STEAPs proteins, and the therapeutic potential of targeting STEAPs for prostate cancer.

Acknowledgement: The authors acknowledge the support from FEDER funds through the POCI - COMPETE 2020 - Operational Programme Competitiveness and Internationalisation in Axis I - Strengthening research, technological development and innovation (Project No. 007491; Project No 029114), National Funds by FCT-Foundation for Science and Technology (Project UID/Multi/00709/2019) and Applied Molecular Biosciences Unit - UCIBIO which is financed by national funds from FCT (UIDP/04378/2020 and UIDB/04378/2020). Sandra M Rocha and Jorge Barroca-Ferreira were funded by PhD Fellowship (SFRH/BD/115693/2016 and SFRH/BD/130068/2017, respectively) and Luís A. Passarinha by sabbatical fellowship (SFRH/BSAB/150376/2019) from FCT.

Conflict of interest: The authors declare no potential conflicts of interest with respect to research, authorship and/or publication of this manuscript.

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REFERENCES

1. Culp MBB, Soerjomataram I, Efstathiou JA, Bray F, Jemal A. Recent Global Patterns in Prostate Cancer Incidence and Mortality Rates. Vol. 77, European Urology. Elsevier B.V.; 2020. p. 38–52. https://doi.org/10.1016/j.eururo.2019.08.005
2. Rawla P. Epidemiology of Prostate Cancer. World J Oncol. 2019;10(2):63–89. https://doi.org/10.14740/wjon1191
3. Cary KC, Cooperberg MR. Biomarkers in prostate cancer surveillance and screening: Past, present, and future. Vol. 5, Therapeutic Advances in Urology. SAGE Publications; 2013. p. 318–29. https://doi.org/10.1177/1756287213495915
4. Gomes IM, Rocha SM, Gaspar C, Alvelos MI, Santos CR, Socorro S, et al. Knockdown of STEAP1 inhibits cell growth and induces apoptosis in LNCaP prostate cancer cells counteracting the effect of androgens. Med Oncol. 2018;35(3). https://doi.org/10.1007/s12032-018-1100-0
5. Grunewald TGP, Diebold I, Esposito I, Plehm S, Hauer K, Thiel U, et al. STEAP1 is associated with the invasive and oxidative stress phenotype of ewing tumors. Mol Cancer Res. 2012 Jan;10(1):52–65. https://doi.org/10.1158/1541-7786.MCR-11-0524
6. Ohgami RS, Campagna DR, McDonald A, Fleming MD. The Steap proteins are metalloreductases. Blood. 2006;108(4):1388–94. https://doi.org/10.1182/blood-2006-02-003681

7. Burnell SEA, Spencer-Harty S, Howarth S, Bodger O, Kynaston H, Morgan C, et al. Utilisation of the STEAP protein family in a diagnostic setting may provide a more comprehensive prognosis of prostate cancer. PLoS One. 2019;14(8):e0220456. https://doi.org/10.1371/journal.pone.0220456
8. Gomes IM, Maia CJ, Santos CR. STEAP proteins: From structure to applications in cancer therapy. Vol. 10, Mol Cancer Res; 2012. p. 573–87. https://doi.org/10.1158/1541-7786.MCR-11-0281
9. Grunewald TGP, Bach H, Cossarizza A, Matsumoto I. The STEAP protein family: Versatile oxidoreductases and targets for cancer immunotherapy with overlapping and distinct cellular functions. Biol Cell. 2012;104(11):641–57. https://doi.org/10.1111/boc.201200027
10. Moreaux J, Kassambara A, Hose D, Klein B. STEAP1 is overexpressed in cancers: A promising therapeutic target. Biochem Biophys Res Commun. 2012;429(3–4):148–55. https://doi.org/10.1016/j.bbrc.2012.10.123
11. Barroca-Ferreira J, Pais JP, Santos MM, Goncalves AM, Gomes IM, Sousa I, et al. Targeting STEAP1 Protein in Human Cancer: Current Trends and Future Challenges. Curr Cancer Drug Targets. 2017;18(3):222–30. https://doi.org/10.2174/1568009617666170427103732
12. Cerami E, Gao J, Dogrusoz U, Gross BE, Sumer SO, Aksoy BA, et al. The cBio Cancer Genomics Portal: An open platform for exploring multidimensional cancer genomics data. Cancer Discov. 2012;2(5):401–4. https://doi.org/10.1158/2159-8290.CD-12-0095
13. Cortazar AR, Torrano V, Martín-Martín N, Caro-Maldonado A, Camacho L, Hermanova I, et al. Cancertool: A visualization and representation interface to exploit cancer datasets. Cancer Res. 2018;78(21):6320–8. https://doi.org/10.1158/0008-5472.CAN-18-1669
14. Kleven MD, Dlakić M, Lawrence CM. Characterization of a single b-type heme, FAD, and metal binding sites in the transmembrane domain of sixtransmembrane epithelial antigen of the prostate (STEAP) family proteins. J Biol Chem. 2015;290(37):22558–69. https://doi.org/10.1074/jbc.M115.664565
15. Oosterheert W, Reis J, Gros P, Mattevi A. An elegant four-helical fold in NOX and STEAP enzymes facilitates electron transport across biomembranes - Similar vehicle, different destination. Acc Chem Res. 2020;53(9):1969–80. https://doi.org/10.1021/acs.accounts.0c00400
16. Hubert RS, Vivanco I, Chen E, Rastegar S, Leong K, Mitchell SC, et al. STEAP: A prostate-specific cell-surface antigen highly expressed in human prostate tumors. Proc Natl Acad Sci U S A. 1999;96(25):14523–8. https://doi.org/10.1073/pnas.96.25.14523
17. Knutson MD. Steap Proteins: Implications for Iron and Copper Metabolism. Nutr Rev. 2008;65(7):335–40. https://doi.org/10.1111/j.1753-4887.2007.tb00311.x
18. Kim K, Mitra S, Wu G, Berka V, Song J, Yu Y, et al. Six-Transmembrane Epithelial Antigen of Prostate 1 (STEAP1) Has a Single b Heme and Is Capable of Reducing Metal Ion Complexes and Oxygen. Biochemistry. 2016;55(48):6673–84. https://doi.org/10.1021/acs.biochem.6b00610
19. Yamamoto T, Tamura Y, Kobayashi J ichi, Kamiguchi K, Hirohashi Y, Miyazaki A, et al. Six-transmembrane epithelial antigen of the prostate-1 plays a role for in vivo tumor growth via intercellular communication. Exp Cell Res. 2013;319(17):2617–26. https://doi.org/10.1016/j.yexcr.2013.07.025
20. Esmaeili S-A, Nejatollahi F, Sahebkar A. Inhibition of Intercellular Communication between Prostate Cancer Cells by A Specific Anti-STEAP-1 Single Chain Antibody. Anticancer Agents Med Chem. 2017;18(12):1674–9. https://doi.org/10.2174/1871520618666171208092115
21. Oosterheert W, Gros P. Cryo-electron microscopy structure and potential enzymatic function of human six-transmembrane epithelial antigen of the prostate 1 (STEAP1). J Biol Chem. 2020;295(28):9502–12. https://doi.org/10.1074/jbc.RA120.013690
22. Oosterheert W, van Bezouwen LS, Rodenburg RNP, Granneman J, Förster F, Mattevi A, et al. Cryo-EM structures of human STEAP4 reveal mechanism of iron(III) reduction. Nat Commun. 2018;9(1). https://doi.org/10.1038/s41467-018-06817-7
23. Schober SJ, Thiede M, Gassmann H, Prexler C, Xue B, Schirmer D, et al. MHC Class I-Restricted TCR-Transgenic CD4+ T Cells Against STEAP1 Mediate Local Tumor Control of Ewing Sarcoma In Vivo. Cells. 2020;9(7):1581. https://doi.org/10.3390/cells9071581
24. Danila DC, Szmulewitz RZ, Vaishampayan U, Higano CS, Baron AD, Gilbert HN, et al. Phase i Study of DSTP3086S, an antibody-drug conjugate targeting six-transmembrane epithelial antigen of prostate 1, in metastatic castration-resistant prostate cancer. J Clin Oncol. 2019;37(36):3518–27. https://doi.org/10.1200/JCO.19.00646

25. Carrasquillo JA, Fine BM, Pandit-Taskar N, Larson SM, Fleming SE, Fox JJ, et al. Imaging patients with metastatic castration-resistant prostate cancer using 89Zr-DFO-MSTP2109A anti-STEAP1 antibody. J Nucl Med. 2019;60(11):1517–23. https://doi.org/10.2967/jnumed.118.222844
26. Jiao Z, Huang L, Sun J, Xie J, Wang T, Yin X, et al. Six-transmembrane epithelial antigen of the prostate 1 expression promotes ovarian cancer metastasis by aiding progression of epithelial-to-mesenchymal transition. Histochem Cell Biol. 2020. https://doi.org/10.1007/s00418-020-01877-7
27. Ihlaseh-Catalano SM, Drigo SA, de Jesus CMN, Domingues MAC, Trindade Filho JCS, de Camargo JL V., et al. STEAP1 protein overexpression is an independent marker for biochemical recurrence in prostate carcinoma. Histopathology. 2013;63(5):678–85. https://doi.org/10.1111/his.12226
28. Gomes IM, Arinto P, Lopes C, Santos CR, Maia CJ. STEAP1 is overexpressed in prostate cancer and prostatic intraepithelial neoplasia lesions, and it is positively associated with Gleason score. Urol Oncol Semin Orig Investig. 2014;32(1):53.e23–53.e29. https://doi.org/10.1016/j.urolonc.2013.08.028
29. Yu-zhuo Pan, Yang Li, Li-rong Guo, Yan-ying Zhao XZ. Influence of expression of six transmembrane epithelial antigen of the prostate-1 on intracellular reactive oxygen species level and cell growth: an in vitro experiment. Zhonghua Yi Xue Za Zhi . 2008;88(9):641–4.
30. Cheung IY, Feng Y, Danis K, Shukla N, Meyers P, Ladanyi M, et al. Novel markers of subclinical disease for Ewing family tumors from gene expression profiling. Clin Cancer Res. 2007;13(23):6978–83. https://doi.org/10.1158/1078-0432.CCR-07-1417
31. Korkmaz KS, Elbi C, Korkmaz CG, Loda M, Hager GL, Saatcioglu F. Molecular cloning and characterization of STAMP1, a highly prostate-specific six transmembrane protein that is overexpressed in prostate cancer. J Biol Chem. 2002;277(39):36689–96. https://doi.org/10.1074/jbc.M202414200
32. Porkka KP, Helenius MA, Visakorpi T. Cloning and characterization of a novel six-transmembrane protein STEAP2, expressed in normal and malignant prostate. Lab Investig. 2002;82(11):1573–82. https://doi.org/10.1097/01.LAB.0000038554.26102.C6
33. Wang L, Jin Y, Arnoldussen YJ, Jonson I, Qu S, Mælandsmo GM, et al. STAMP1 is both a proliferative and an antiapoptotic factor in prostate cancer. Cancer Res. 2010;70(14):5818–28. https://doi.org/10.1158/0008-5472.CAN-09-4697
34. Whiteland H, Spencer-Harty S, Morgan C, Kynaston H, Thomas DH, Bose P, et al. A role for STEAP2 in prostate cancer progression. Clin Exp Metastasis. 2014;31(8):909–20. https://doi.org/10.1007/s10585-014-9679-9
35. Burnell SEA, Spencer-Harty S, Howarth S, Bodger O, Kynaston H, Morgan C, et al. STEAP2 Knockdown Reduces the Invasive Potential of Prostate Cancer Cells. Sci Rep. 2018 Dec 1;8(1). https://doi.org/10.1038/s41598-018-24655-x
36. Ohgami RS, Campagna DR, Greer EL, Antiochos B, McDonald A, Chen J, et al. Identification of a ferrireductase required for efficient transferrin-dependent iron uptake in erythroid cells. Nat Genet. 2005;37(11):1264–9. https://doi.org/10.1038/ng1658
37. Sendamarai AK, Ohgami RS, Fleming MD, Lawrence CM. Structure of the membrane proximal oxidoreductase domain of human Steap3, the dominant ferrireductase of the erythroid transferrin cycle. Proc Natl Acad Sci U S A. 2008;105(21):7410–5. https://doi.org/10.1073/pnas.0801318105
38. Lespagnol A, Duflaut D, Beekman C, Blanc L, Fiucci G, Marine JC, et al. Exosome secretion, including the DNA damage-induced p53-dependent secretory pathway, is severely compromised in TSAP6/Steap3-null mice. Cell Death Differ. 2008;15(11):1723–33. https://doi.org/10.1038/cdd.2008.104
39. Wan C, Fu J, Wang Y, Miao S, Song W, Wang L. Exosome-related multi-pass transmembrane protein TSAP6 is a target of rhomboid protease RHBDD1-induced proteolysis. PLoS One. 2012;7(5). https://doi.org/10.1371/journal.pone.0037452
40. Wilkinson HN, Upson SE, Banyard KL, Knight R, Mace KA, Hardman MJ. Reduced Iron in Diabetic Wounds: An Oxidative Stress-Dependent Role for STEAP3 in Extracellular Matrix Deposition and Remodeling. J Invest Dermatol. 2019;139(11):2368–2377.e7. https://doi.org/10.1016/j.jid.2019.05.014
41. Zhang F, Tao Y, Zhang Z, Guo X, An P, Shen Y, et al. Metalloreductase steap3 coordinates the regulation of iron homeostasis and inflammatory responses. Haematologica. 2012;97(12):1826–35. https://doi.org/10.3324/haematol.2012.063974

42. Feng Z. p53 regulation of the IGF-1/AKT/mTOR pathways and the endosomal compartment. [Internet]. Vol. 2, Cold Spring Harbor perspectives in biology. Cold Spring Harb Perspect Biol; 2010. https://doi.org/10.1101/cshperspect.a001057
43. Zhang X, Steiner MS, Rinaldy A, Lu Y. Apoptosis induction in prostate cancer cells by a novel gene product, pHyde, involves caspase-3. Oncogene. 2001;20(42):5982–90. https://doi.org/10.1038/sj.onc.1204831
44. M S Steiner, X Zhang, Y Wang YL. Growth inhibition of prostate cancer by an adenovirus expressing a novel tumor suppressor gene, pHyde. Cancer Res . 2000;60(16):4419–25.
45. Porkka KP, Nupponen NN, Tammela TLJ, Vessella RL, Visakorpi T. Human pHyde is not a classical tumor suppressor gene in prostate cancer. Int J Cancer. 2003;106(5):729–35. https://doi.org/10.1002/ijc.11278
46. Korkmaz CG, Korkmaz KS, Kurys P, Elbi C, Wang L, Klokk TI, et al. Molecular cloning and characterization of STAMP2, an androgen-regulated six transmembrane protein that is overexpressed in prostate cancer. Oncogene. 2005;24(31):4934–45. https://doi.org/10.1038/sj.onc.1208677
47. Scarl RT, Lawrence CM, Gordon HM, Nunemaker CS. STEAP4: Its emerging role in metabolism and homeostasis of cellular iron and copper. Vol. 234, Journal of Endocrinology. 2017. p. R123–34. https://doi.org/10.1530/JOE-16-0594
48. Jin Y, Wang L, Qu S, Sheng X, Kristian A, Mælandsmo GM, et al. STAMP 2 increases oxidative stress and is critical for prostate cancer . EMBO Mol Med. 2015;7(3):315–31. https://doi.org/10.15252/emmm.201404181
49. Tamura T, Chiba J. STEAP4 regulates focal adhesion kinase activation and CpG motifs within STEAP4 promoter region are frequently methylatedin DU145, human androgen-independent prostate cancer cells. Int J Mol Med. 2009;24(5):599–604. https://doi.org/10.3892/ijmm_00000270
50. Wang Q, Tiffen J, Bailey CG, Lehman ML, Ritchie W, Fazli L, et al. Targeting amino acid transport in metastatic castration-resistant prostate cancer: Effects on cell cycle, cell growth, and tumor development. J Natl Cancer Inst. 2013;105(19):1463–73. https://doi.org/10.1093/jnci/djt241
51. Taylor BS, Schultz N, Hieronymus H, Gopalan A, Xiao Y, Carver BS, et al. Integrative Genomic Profiling of Human Prostate Cancer. Cancer Cell. 2010;18(1):11–22. https://doi.org/10.1016/j.ccr.2010.05.026
52. Grasso CS, Wu YM, Robinson DR, Cao X, Dhanasekaran SM, Khan AP, et al. The mutational landscape of lethal castration-resistant prostate cancer. Nature. 2012;487(7406):239–43. https://doi.org/10.1038/nature11125
53. Tomlins SA, Mehra R, Rhodes DR, Cao X, Wang L, Dhanasekaran SM, et al. Integrative molecular concept modeling of prostate cancer progression. Nat Genet. 2007;39(1):41–51. https://doi.org/10.1038/ng1935
54. Varambally S, Yu J, Laxman B, Rhodes DR, Mehra R, Tomlins SA, et al. Integrative genomic and proteomic analysis of prostate cancer reveals signatures of metastatic progression. Cancer Cell. 2005;8(5):393–406. https://doi.org/10.1016/j.ccr.2005.10.001
55. Lapointe J, Li C, Higgins JP, Van De Rijn M, Bair E, Montgomery K, et al. Gene expression profiling identifies clinically relevant subtypes of prostate cancer. Proc Natl Acad Sci U S A. 2004;101(3):811–6. https://doi.org/10.1073/pnas.0304146101
56. Wu HT, Chen WJ, Xu Y, Shen JX, Chen WT, Liu J. The Tumor Suppressive Roles and Prognostic Values of STEAP Family Members in Breast Cancer. Biomed Res Int. 2020. https://doi.org/10.1155/2020/9578484
57. Chen H, Xu C, Yu Q, Zhong C, Peng Y, Chen J, et al. Comprehensive landscape of STEAP family functions and prognostic prediction value in glioblastoma. J Cell Physiol. 2020;jcp.30060. https://doi.org/10.1002/jcp.30060
58. Prasad B, Tian Y, Li X. Large-Scale Analysis Reveals Gene Signature for Survival Prediction in Primary Glioblastoma. Mol Neurobiol. 2020;57(12):5235–46. https://doi.org/10.1007/s12035-020-02088-w
59. Liang Y, Xing X, Beamer MA, Swindell WR, Sarkar MK, Roberts LW, et al. Six-transmembrane epithelial antigens of the prostate comprise a novel inflammatory nexus in patients with pustular skin disorders. J Allergy Clin Immunol. 2017;139(4):1217–27. https://doi.org/10.1016/j.jaci.2016.10.021
60. Nyirongo VB, Mukaka MM, Kalilani-Phiri L V. Statistical pitfalls in medical research. Malawi Med J. 2008;20(1). https://doi.org/10.4314/mmj.v20i1.10949

Index

Doi: https://doi.org/10.36255/exonpublications.prostatecancer.index.2021

Prostate Cancer

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ISBN 978-0-645-00175-4

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