THE UNIVERSITY OF CHICAGO

COORDINATED ACTIVITY BETWEEN AR-V7 AND GLUCOCORTICOID RECEPTOR IN CASTRATION-RESISTANT PROSTATE CANCER

A DISSERTATION SUBMITTED TO

THE FACULTY OF THE DIVISION OF THE BIOLOGICAL SCIENCES

AND THE PRITZKER SCHOOL OF MEDICINE

IN CANDIDACY FOR THE DEGREE OF

DOCTOR OF PHILOSOPHY

COMMITTEE ON CANCER BIOLOGY

BY

ERIC CHARLES BUETER

CHICAGO, ILLINOIS

AUGUST 2022

for the boys

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

ACKNOWLEDGEMENTS

First, I need to thank my mentor, Russell Szmulewitz. This was an incredibly challenging project, and his unwavering support throughout was instrumental in seeing it through to the finish. I am also grateful for the support of my committee: Geoffrey Greene, Suzanne Conzen, and Stephen Kron. Each played an essential role in shaping the direction of the project and helping my personal development as a scientist. Perhaps no individual was more influential in my development as a scientist as Anthony Williams, whose mentorship I will treasure. Many other members of the Szmulewitz lab contributed to this work, including Megan Mendez, Ari Halle, Chuck Blajszczak, Dave Hosfield, Jaime Barraza, Erin McAuley, Tiha Long, Ryan Brown, Shane Comiskey, and, most notably, Phillip Selman. I received invaluable support during the project from Vytas Bindokas at the University of Chicago Microscopy core, Terry Li at the University of Chicago Human Tissue Resource Center, and Pieter Faber at the University of Chicago Genomics Facility as well. Additional faculty to whom I owe a debt of gratitude include Kay Macleod, Marsha Rosner, and Don Vander Griend.

Throughout this journey, my family has been unconditionally supportive as I've worked through the struggles of graduate school. I want them to know that I feel so loved every day and without their support I would never have made it this far. I also need to thank the teachers and the mentors who have helped me reach this point, including but not limited to: Kara Petras, Maryellen Knight, Jim Sauer, Charlie Furman, Carrie Smith, Mary Lord, Mary Lewis, Ray Kuhn, Christa Colyer, Rebecca Alexander, Paul Jedlicka, Scott Potosky, Greg Gidman, Dave Harr, and so many others who inspired me to take this journey. I am blessed with the best group of friends a man could ever ask for. David, DJ, Emerson, Andrew, Corey, Evan, Nick, Alex,

Taylor, Audrey, Ben, Fletcher, Mike, Ryan, Bennett, Vingi, Layne, Todd, KJ, Crotty, Timmy, Keshian, Hickman, RyMo, and a bunch more who I'm forgetting but are almost certainly never going to read this. I am truly grateful for you all. Finally, I don't think I would ever have been here if it were not for Sean. Wherever you are buddy, I hope you're proud. I miss you every single day.

ABSTRACT

Acquired resistance to androgen receptor signaling inhibition (ARSI) is a major clinical challenge for treating advanced prostate cancer. Both the glucocorticoid receptor (GR) and the androgen receptor (AR) splice variant AR-V7 have independently been identified as drivers of this phenomenon. Their mechanisms of resistance share many common features, including upregulation following ARSI and the ability to bypass AR to regulate canonical AR signaling and restore tumor progression. However, despite the similarities no direct relationship between the two has been investigated. Here, we report a novel endogenous interaction between GR and AR-V7 in prostate cancer cell lines. The interaction is mediated by the conserved D-box motif within the DNA-binding domain of each receptor, suggesting heterodimerization. Furthermore, the interaction is only observed subsequent to ARSI, occurs primarily within the nucleus, and enables GR and AR-V7 to co-occupy the promoter of the canonical AR target gene FKBP5. Robust transcriptome analysis reveals that AR-V7 and GR coordinate to drive a unique gene expression profile enriched for proliferative and anti-apoptotic signaling pathways. Finally, we demonstrate that selective glucocorticoid receptor modulators can effectively disrupt the interaction between GR and AR-V7, indicating a potential therapeutic strategy for the subset of V7/GR dual-positive patients with very poor prognoses.

CHAPTER 1

INTRODUCTION

1.1 HISTORICAL PERSPECTIVES OF THE PROSTATE

The prostate is an exocrine secretory gland of the male reproductive system unique to mammals [l]. The earliest historical mention of the prostate can be traced back to the third century BC by the Greek physician Herophilus of Chalcedon, who described *parastatai adenoneides* or glandular assistants [2]. Nearly three hundred years later, Galen of Pergamum expanded on Herophilus' work to describe glands which 'poured a humor into the urinary passages of the male to excite the sexual act' [2]. This limited description of the prostate remained the status quo for several centuries, until the Renaissance period in Europe reignited interest in scientific discovery. Italian scientist Niccolò Massa re-identified a 'glandular flesh upon which rests the neck of the bladder' in 1536 and the first illustration of the prostate was subsequently published by Flemish scientist Andreas Vesalius in 1538 [3, 4]. The term prostate, derived from Herophilus' *parastatai*, was formally introduced later in the 16th century [5].

In humans, the prostate is located in the pelvis, and sits below the bladder surrounding the urethra [1]. Pathologist John McNeil divided the prostate into three distinct anatomical zones, each with different embryologic origins and unique biological functions. The peripheral zone (PZ), made up of duct buds that develop laterally surrounding the distal urethra, accounts for about 70% of the glandular tissue in the prostate [6]. The central zone (CZ) makes up another 25% of the glandular tissue, and its ducts bud proximally into the ejaculatory ducts [6]. The transition zone (TZ) surrounds the proximal urethra and accounts for the remaining 5% of glandular tissue [6]. The final component of the prostate is non-glandular anterior fibro-muscular stroma, which forms

an apron to shield the glandular components of the prostate [6]. About 70% of prostate cancer (PC) arises in the PZ, while another 25-30% originates in the TZ [1]. Less than 5% of cancers are observed in the CZ [1]. The concentration of PC in the PZ and TZ can be attributed to their embryonic origins in the urogenital sinus [1]. Comparatively, the TZ is derived from Wolffian duct ancestry, whose matured organs, notably the seminal vesicles, display very low rates of carcinogenesis [1].

The human prostate plays several important physiological roles in the maintenance and function of the male reproductive program. Secretory prostatic fluids make up between 20-30% the total volume of semen and contribute towards maintaining a favorable microenvironment necessary for sperm to survive to fertilization [7]. When the prostate contracts to secrete fluids during ejaculation, the connection between the bladder and the urethra is also blocked, preventing simultaneous urination and ejaculation [8]. The highly innervated prostate has also been suggested to contribute to the feeling of ecstasy experienced during orgasm, with prostatic stimulation alone being sufficient to achieve orgasm in some men [9]. As part of the male reproductive program, the prostate requires androgens to maintain full development and functionality [10]. Loss of the ability to properly regulate androgen signaling in the prostate is a hallmark of prostate disease, including cancer [1]

English physician J. Adams documented the first case of PC established by histology at The London Hospital in 1853 [11]. However, in subsequent decades PC incidence was sporadic and rare [12]. Around the turn of the century, novel surgical techniques allowed for easier tissue sample collection and histological analysis. This development significantly increased the incidence of PC and consequently the demand for therapy [11]. In 1904, Hugh Hampton Young pioneered the first radical perineal prostatectomy, a revolutionary surgery that would become the

standard surgical procedure for over 40 years [13]. Four decades later, Irish surgeon Terrence Millin introduced an improved method of prostatectomy, the retropubic approach for prostate enucleation, which became the standard of care for the following 40 years [13]. However, despite the known benefits of surgery, many patients during this time refused operations because in most instances the procedure would result in impotence, necessitating alternative forms of therapy [13].

Eight years before Young performed the first radical peritoneal prostatectomy, German physicist Wilhelm Conrad Roentgen made the remarkable discovery of X-rays in 1896 [14]. Two years later, Marie Curie and colleagues had described the radioactive properties of radium [14]. Within a decade, radiation therapy was being trialed as a potential cancer therapeutic and both Roentgen and Curie would be awarded Nobel prizes for revolutionizing the field of oncology [14]. Early prostate radiotherapy consisted of the introduction of radium sources into the urethra or rectum [15]. Eventually, techniques evolved to deliver radium via needles directly to the prostate gland via the peritoneum [16]. While these approaches demonstrated variable efficacy in reducing tumor burden, they would frequently come at great discomfort to the patient, again highlighting the need for better therapeutic strategies in the 20th century and beyond [16].

The link between androgens and the prostate was first considered by surgeon John Hunter in 1786, when he observed seasonal changes to the size of the testicles and prostates in deer, mice, and moles [17]. In subsequent experiments with castration, Hunter established a direct connection between the testes and secondary sex organs [17]. However, it wasn't for another 100 years, when PC incidence began to rise, that Hunter's work was revisited. In 1893, Philadelphia surgeon W. White became the first to advocate for castration as a treatment for urinary obstruction disorders, after he observed atrophy in the glandular tissue of the dog prostate following castration [11]. Over several years of castrating patients, White generated mixed results, with some procedures resulting

in significant atrophy of the prostate and others having little effect [11]. Along similar lines, Robert Moore and Allister McLellan experimented injecting patients with estrogen and similarly observed atrophy of the prostate [18]. In 1938, husband and wife Ethel and Alexander Gutman detected increased serum acid-phosphatase levels in patients with metastatic prostate cancer (mPC) [19]. These landmark discoveries would provide a foundation for Charles' Huggins landmark Nobel Prize winning work.

Huggins utilized the levels of the serum acid-phosphatases observed by Gutman and Gutman as a proxy to measure hormonal activity [20]. He then demonstrated either castration or the administration of estrogen caused glandular atrophy and decreases in serum acid-phosphatases [20]. Furthermore, Huggins established that this effect could be reversed by re-administering androgens [20]. In 1941, Huggins published his seminal work demonstrating the therapeutic benefit of castration or estrogen administration in patients with PC [21]. This discovery established androgen receptor signaling inhibition (ARSI) as the foundation for all PC treatment in the 20th century and beyond.

1.2 PROSTATE CANCER INCIDENCE AND RISK

PC is the most common cancer diagnosis among men worldwide and ranks in the top five of all cancers for both incidence and mortality [22]. An estimated 1.5 million men are diagnosed with PC annually, with roughly 375,000 individuals succumbing to the disease each year [22]. In 2021, PC was responsible for 7.3% of all cancers and 3.8% of all cancer death worldwide [23]. As populations continue to grow and age, the global burden of PC is projected to reach 1.7 million cases and 500,000 deaths by 2030 [24]. In the United States, PC is the most commonly diagnosed cancer and second leading cause of cancer death among men, with an estimated 268,490 new cases

diagnosed and 34,500 deaths expected in 2022 [25]. The American Cancer society estimates 1 in 8 American men will be diagnosed with PC in their lifetime [25].

PC is notable for its striking geographical variation in both cases and fatalities, with incidence rates varying up to 30-fold between selected registries and mortality rates varying up to 18-fold [26]. Despite holding less than 20% of the global population, developed nations (Europe, North America, Australia, New Zealand, Japan) account for 72% of new PC cases and 53% of PC death [27]. While much of this discrepancy is attributed to better access to healthcare and more intensive screening strategies in developed countries, particular demographic groups remain at risk [26]. Men of African descent are particularly vulnerable to PC, with the highest mortality rates observed in Afro-Caribbean, sub-Saharan African, and African American populations [26]. Just within the United States, African American men die of PC at more than double the rate of their white counterparts [28]. At the other end of the spectrum, men with Asian or North African ancestry experience the lowest rates of PC incidence and mortality, particularly those still living in their native countries [26].

Age is the risk factor most strongly associated with PC [25]. While PC is rarely diagnosed in men under 40, the chance of having PC begins rising rapidly beginning after the age of 50 [25]. Roughly 6 in 10 cases of PC occur in patients over the age of 65 and the average age of PC diagnosis is 66 [25]. Like in many other cancers, diet and exercise are also strong risk factors for PC. Individuals with high fat diets, particularly polyunsaturated fats, experienced higher rates of PC incidence and mortality [29]. Conversely, men that reported exercising regularly demonstrated significantly reduced rates of PC incidence and mortality [30]. Consistent with this, the administration of statins, a lipid-lowering class of drugs, has consistently shown an inverse relationship with PC progression [31]. Excessive consumption of alcohol, tobacco, and dairy

products has been associated with increased risk for PC, while consumption of coffee, tomatoes, and fish has been associated with decreased risk; however, these effects are less robustly observed [29]. PC also has a strong familial component, with the highest reported heritability among major cancers [32]. Studies performed in twins estimate that 57% of variation in risk can be attributed to genetic factors [32].

Several recurring non-hereditary genetic abnormalities have been observed in PC [33]. Androgen signaling plays a crucial role in regulating prostate growth and the majority of primary and metastatic PCs harbor abnormalities in this pathway, including AR amplification, gain of function mutations, and the expression of splice variants [33]. Many tumors also display elevated levels of AR coactivators or decreased levels of AR corepressors [33]. Aberrations to the master regulator p53 are observed in over half of all mPC [33]. One frequent genomic alteration observed in PC involves the fusion of an androgen-regulated promoter to a member of the highly oncogenic ETS family of transcription factors, exemplified by the TMPRSS2:ERG fusion found in ~50% of all primary PCs [33,34]. Coupling ERG expression to AR signaling causes *ERG* overexpression, which is sufficient for oncogenic transformation in epithelial cells [35]. The PI3K kinase pathway is an upstream regulator of AR, and dysregulation of this pathway is frequently seen in PC where PTEN loss is observed in nearly 40% of tumors [33].

1.3 CONTEMPORARY PROSTATE CANCER THERAPIES

Early detection of PC is essential for efficient therapeutic interventions. The digital rectal exam has historically been used to physically detect the presence of cancer in the PZ of the prostate [36]. In 1987, Stamey and colleagues published a landmark study analyzing 2200 serum samples from 699 patients, 378 of whom had PC and 321 who did not. They found that the level of prostate specific antigen (PSA) was proportional to the estimated size of tumors and correlated with

pathological stage of PC [37]. Furthermore, it was demonstrated that PSA was undetectable following radical prostatectomy [37]. Four years later Catalona and colleagues successfully trialed PSA as a marker to screen for PC, establishing a standard assay for PC screening still used today [38]; however, the implementation of PSA screening at the population level has been somewhat controversial. The 4ng/mL cutoff used clinically only has a calculated sensitivity of 78.7% and calculated specificity of 59.2% [39]. Adjusting the threshold to 5ng/mL raises the specificity to 95%, however the sensitivity drops to only 33%, inadequate for population level screening [39]. Opponents of PSA screening at 4ng/mL point to the overdiagnosis and overtreatment associated with the significant number of false positives while proponents of PSA testing will point to the significant reduction in overall PC mortality following its introduction [40]. Implementation of PSA screening has reduced the mean age of diagnosis by nearly 6 years in the United States and shifted pathologic staging towards fewer men being diagnosed with advanced disease [40]. Importantly, PSA testing alone is insufficient to diagnose PC.

If a physician suspects a patient has PC, the most common diagnostic method is through biopsy [41]. Transrectal ultrasound imaging is used to identify areas of the vulnerable prostate to PC and several core needle biopsies are acquired [42]. Samples are then sent to a pathologist to be assigned a Gleason score, a methodology for grading tumors unique to PC [43]. Gleason scoring relies on five distinct states of PC as they transform from normal cells to tumor cells. The two patterns most predominantly observed in each biopsy are identified and added together to achieve the Gleason score. While Gleason scores theoretically range from 2-10, a Gleason score of 6 is associated with the lowest grade of PC [43]. Gleason 7 scores are considered intermediate grade, although Gleason 4+3 tumors display more aggressive phenotypes than Gleason 3+4 tumors [43].

Receiving a Gleason score of 8 or above is indicative of high-grade disease and poor prognosis [43].

Therapeutic intervention for treating PC varies considerably on a patient-to-patient basis [41]. Patients that present with localized disease, accounting for roughly three quarters of clinical cases, have a 5-year survival rate of nearly 100% [25]. For these individuals, surgery and radiation are the primary treatment option [41]. Some asymptomatic patients with indolent tumors do not require any treatment at all, with certain individuals instead opting for active surveillance and watchful waiting to determine if the tumor grows to a level that requires medical intervention in the future [44]. Unfortunately, more aggressive therapeutic strategies are required for patients with advanced PC, as the 5-year survival rate for this group drops to only 28% [25].

Following Huggins' revolutionary discoveries in the mid-20th century, androgen deprivation therapy (ADT) emerged as the treatment of choice for PC [11]. The first line of therapy for patients with advanced PC is typically castration, either surgical or chemical, to reduce systemic androgen levels in the body [41]. Historically, circulating levels of androgens in the bloodstream have been used to assess the efficacy of castration, with target testosterone levels below 50ng/dL [45]. Surgical castration is achieved through bilateral orchiectomy, removing the testes where Leydig cells produce about 90% of circulating androgens in the body [46]. Huggins first demonstrated the efficacy of surgical castration reducing disease burden in 1941, and it quickly became the standard of care [21]. Building on Huggins' success with surgical castration, other groups investigated alternative mechanisms of androgen deprivation that avoid the disfiguration associated with surgery. Chemical castration was pioneered by Polish endocrinologist Andrew Schally, who synthesized and characterized the hypothalamic luteinizing hormone (LH)-releasing hormone (LHRH) in 1971 [47]. Hypothalamic release of LHRH

stimulates the production and release of LH in gonadotropic cells of the pituitary gland, and LH binds receptors on the testes to stimulate androgen synthesis [47]. Schally was able to manipulate this system with synthetic agonists and antagonists of LHRH and observed that prolonged exposure to these molecules initiated a negative feedback loop that lowered the concentration of androgens in the body to castrate levels [47]. By 1977, Schally had earned a Nobel Prize in Physiology and Medicine and chemical castration was prevalent in hospitals across the country [11]. Several subsequent studies comparing the effectiveness of surgical castration to chemical castration did not find meaningful differences [48]. Castration prolongs overall survival, produces an objective response in metastatic lesions, relieves associated pain, and suppresses PSA levels in roughly 80% of patients [41]. While tumors exhibit a dramatic response initially, resistance is inevitable as residual androgens in the body drive disease progression [49].

Castration-resistant prostate cancer (CRPC) is the lethal form of the disease, and progression to this stage is considered terminal [41]. Interestingly, tumors at this stage typically are still driven by residual androgen signaling and remain responsive to compounds targeting the androgen receptor (AR) [49]. In 1989, the FDA approved flutamide the first non-steroidal antiandrogen, followed by nilutamide in 1996 and bicalutamide in 2008 [50,51,52]. These first-generation antiandrogens selectively compete to bind AR and effectively prevent nuclear translocation [53]. However, a major shortcoming of these compounds is that when AR is present at sufficiently high levels, there is a switch from antagonist to agonist, rendering them ineffective in many patients and leading to the development of second-generation antiandrogens [54].

Enzalutamide (enz) is a second-generation antiandrogen approved by the FDA for use in PC in 2012. Like its predecessors, enz is non-steroidal and directly targets the AR ligand-binding domain (LBD) as a selective competitor [55]. However, in addition to preventing nuclear

translocation, enz also impairs the ability of AR to bind DNA and recruit coactivators [55]. The first major phase III clinical trial to demonstrate the efficacy of enz was the 2012 AFFIRM study, utilizing a cohort of patients with metastatic CRPC (mCRPC) previously treated with chemotherapy [56]. This was followed by the 2014 PREVAIL trial in a cohort chemotherapy-naïve patients with mCRPC [57]. Finally, the 2018 PROSPER study examined enz in a cohort of patients with non-metastatic CRPC [58]. In each trial, enz significantly prolonged progression free survival and overall survival among other benefits. The success of enz led to the development of two new non-steroidal antiandrogens approved by the FDA in 2019 for treating non-metastatic CRPC: darolutamide and apalutamide [59]. In two major studies, ARAMIS (darolutamide) and SPARTAN (apalutamide), these compounds were able to delay median time for metastasis-free survival from 16 months to around 40 months compared to placebo [60,61]. Network meta-analysis suggests apalutamide and enz are superior to darolutamide in achieving metastasis-free response and PSA progression free survival [59]. However, darolutamide was posited to be the safest with the fewest number of adverse effects [59].

The second-generation antiandrogen abiraterone (abi) first received FDA approval in 2011. Unlike its counterparts, abi does not directly target the AR LBD [62]. Instead, abi irreversibly inhibits CYP17A1, a critical enzyme in androgen biosynthesis that is expressed in testicular, adrenal, and prostatic tissues [62]. CYP17A1 levels are on average over 10-fold higher in CRPC metastases compared to primary tumors and allow PC cells to locally synthesize their own androgens for maintaining growth [63]. Treatment with abi lowers testosterone levels up to 90% relative to castrate levels, creating an androgen-depleted microenvironment for tumors to grow in [64]. Importantly, abi also inhibits a critical step in glucocorticoid (GC) biosynthesis and is administered in combination with prednisone as means of glucocorticoid replacement [64].

Several alternative strategies for targeting AR signaling are currently being studied. Following the clinical success of the selective estrogen receptor modulator tamoxifen for treating breast cancer, researchers began investigating whether similar selective AR modulators (SARMs) could be viable for treating PC. The notable characteristic of SARMs is their tissue specificity, inducing different responses dependent on which tissue of the body it is acting [65]. SARM development was pursued by several pharmaceutical companies in the late 1990s and early 2000s, and several lead compounds demonstrated potent tissue-selectivity and anabolic activity [65]. However, these compounds failed to advance through clinical trials either due to toxicity or lack of efficacy [65]. Another class of drugs, selective AR degraders (SARDs), utilize various mechanisms to designate AR for proteolysis, thus preventing its oncogenic transcriptional activity [66]. The steroidal antiandrogen galeterone is able to bind the AR LBD directly to induce degradation [67]. A more precise approach involves the use of proteolysis-targeting chimeras (PROTACs) [68]. These heterobifunctional small molecules consist of a central linker connecting two “warheads”, one of which targets the AR LBD while the other recruits E3 ubiquitin ligase, allowing for efficient destruction of AR via the proteasome [68]. Preclinical studies with SARDs demonstrate strong anti-tumor activity [69,70]. However, these compounds have failed in phase III clinical trials due to poor bioavailability [66]. A less conventional approach has been to target dimerization to prevent AR transcriptional activity. AR dimerization is mediated by a highly conserved D-box motif within the DNA-binding domain (DBD), and small molecules disrupting this interaction show strong antineoplastic activity [71]. This target is of particular importance because it is unaffected by traditional mechanisms of cross-resistance that affect antiandrogens.

Pure antiandrogen therapy alone has consistently been found inferior to castration in terms of overall and progression-free survival [41]. However, combining multiple methods of AR

inhibition has emerged as a viable therapeutic strategy [11]. This approach was first pioneered by Ferdinand Labrie and colleagues in the 1980s, examining the effects of an LHRH agonist in combination with an antiandrogen [11]. In 1989, a landmark trial of leuprolide in combination with flutamide resulted in a slightly longer progression-free survival, inspiring a wave of additional trials [72]. However, a meta-analysis of 22 subsequent trials using a first-generation antiandrogen in combination with an LHRH agonist concluded combination therapy did not provide significant survival benefit [73]. Combination therapy with second-generation anti-androgens has been more promising. In patients with hormone-sensitive PC, the ENZAMET and ARCHES trials indicated up front enz treatment in combination with castration significantly increased overall survival and progression free survival [75,76]. Similarly, results from the LATITUDE study found increased overall survival in a similar cohort of men with hormone-sensitive PC who received abi in combination with castration [76]. Unsurprisingly, cross-resistance between antiandrogens is frequently observed, as exposure to one compromises the effectiveness of others, with tumors developing general mechanisms of resistance [77]. Interestingly, the sequence particular antiandrogens are administered can impact their efficacy, with emerging data suggesting the use of abi before enz is associated with better progression free survival and PSA response rates [78].

Acquired resistance to ARSI is a major clinical challenge for the treatment of advanced PC and few treatment options exist for patients whose tumors progress to a hormone-naïve stage [41]. Docetaxel, a chemotherapeutic agent that inhibits microtubule synthesis to impair cell division, was the first drug to prolong life in men with mCRPC and has been considered the standard of care for late-stage disease since 2004 [79]. For the small subset of patients with tumors bearing mutations to DNA repair genes, such as BRCA1/2 or ATM, treatment with PARP inhibitors extends progression-free survival compared to standard therapy alone [80]. While DNA-damaging

chemotherapeutic agents like cis-platin and carboplatin alone have not shown significant benefit in large scale clinical trials, there is emerging evidence that they can be effective for treating patients with impaired DNA repair function [81]. PC is considered to be an immune “cold” malignancy, with minimal immune infiltration observed into the tumor microenvironment [82]. Additionally, the mutational burden of PC is relatively low [83]. These have been posited as reasons for conventional checkpoint immunotherapies failing to display significant benefit in clinical trials [84]. In fact, the only immunotherapeutic to date that demonstrates a survival benefit among men with mCRPC is the cancer vaccine sipuleucel-T, consisting of autologous peripheral-blood mononuclear cells that are activated ex vivo with a recombinant fusion protein, which conferred modest improvement in median survival during the IMPACT trial [85]. Novel therapeutic targets in PC are continuously investigated, and promising candidates include bromodomain inhibitors, EZH2 inhibitors, and ferroptosis inducers [86]. Ultimately, the time to death following progression to hormone-naïve PC is short, highlighting the importance of managing resistance to ARSI [86].

1.4 NUCLEAR HORMONE RECEPTOR BIOLOGY

Understanding nuclear hormone receptor biology is crucial for understanding resistance to ARSI. Hormone receptors are members of the steroid receptor subgroup 3 of the nuclear receptor superfamily (NR3) [87]. Within this subgroup, 3-ketosteroid receptors are classified as part of the NR3C family [87]. These receptors include the glucocorticoid receptor (GR; NR3C1), mineralocorticoid receptor (MR; NR3C2), progesterone receptor (PR; NR3C3), and androgen receptor (AR; NR3C4) [87]. Hormone receptors sense lipid metabolites in the cellular microenvironment to drive differential gene expression resulting in distinct phenotypes [88]. In the absence of ligand, AR and GR primarily reside in the cytoplasm where they are sequestered,

bound to heat shock proteins and other chaperones [89]. Ligand-binding induces a conformational change in the receptor that releases the receptor from its inhibitory complex allowing for nuclear translocation [90]. In the nucleus, NR3C-family receptor homodimers bind specific inverted palindromic repeat sequences called hormone response elements and recruit cofactors to either activate or repress specific gene targets [91]. Between ligand-dependent transcription and a large number of associated coactivators and corepressors, NR3C-family receptors are able to coordinate a massive range of cellular responses dependent on specific conditions in the local microenvironment [90].

The architecture of NR3C-family receptors is modestly conserved and split into five domains from A to E [90]. A/B domains compose the highly variable N-terminal transactivating domain (NTD), which is the target for most post-translational modifications and contains the AF-1 region responsible for interacting with most coregulators [90]. The C domain is the most conserved domain across NR3C-family receptors and contains the DBD [90]. Two subdomains exist within the DBD, each with four cysteine residues coordinating around a zinc ion in a zinc finger motif [90]. The first subdomain, proximal to DNA and naturally referred to as the proximal box or P-box, contains the DNA-reading helix, which interacts with the major groove to make base-specific interactions with the DNA [88]. The second subdomain, the distal box or D-box, makes non-specific contacts with the DNA backbone and contains residues essential for receptor dimerization [88]. The D domain comprises the hinge region, a short flexible linker between the DBD and LBD that contains a nuclear localization signal. Finally, the E domain comprises the LBD, a complex allosteric signaling domain that can bind ligands as well as coregulatory proteins. Generally, twelve alpha-helices and four beta-strands fold into three parallel layers that form an alpha-helical sandwich creating a hydrophobic pocket at the base of the receptor for ligands to

bind [90]. Importantly, other motifs within the LBD are essential for dimerization and help discriminate between different members of the NR3C-family to ensure homodimerization [90].

The primary ligands for AR are the steroid hormones testosterone and dihydrotestosterone, the latter having nearly 3-fold higher affinity for the AR LBD compared to the former [1]. Found on the X-Chromosome (q11-12), eight exons compose the 110kDa full-length androgen receptor (AR-FL). Exon 1 encodes the NTD, exons 2-3 encode the DBD, exon 4 encodes the hinge region, and exons 5-8 encode the LBD [92]. In the prostate, AR expression is tightly regulated by a negative feedback loop, and ARSI results in an upregulation of AR expression [93]. Excessive levels of AR transcript can overload cellular splicing machinery resulting in the expression of several AR splice variants [94]. To date, at least 22 AR splice variants have been described, and all but one involves truncations to the C-terminal LBD [94]. In some cases, variants arise from exon 3 splicing to cryptic exons located in AR intron 3 [95]. Because the expression of AR splice variants is tied to irregular AR transcription, AR splice variants are commonly found in PC [33]. However, the biological prevalence and impact of splice variants varies considerably [94].

The most pervasive and well-studied AR splice variant is AR-V7. It was first detected by Clifford Tepper in 2002, and functionally characterized by Scott Dehm in 2008 [96,97]. Made up of exons 1-3 spliced to cryptic exon 3, AR-V7 loses its LBD but retains a functional N-terminal trans-activating domain and DBD [97]. Notably, despite lacking the canonical nuclear localization sequence found in the AR hinge region, AR-V7 is able to localize to the nucleus and confer constitutive androgen-independent transcriptional activity [97,98,99]. Like other NR3C-family receptors, dimerization is a requirement for AR-V7 function [100]. However, AR-V7 has demonstrated the unique ability to homodimerize as well as heterodimerize with AR-FL in a canonical D-box dependent manner [100].

GR, found in most tissues in the body, is the most ubiquitous of the NR3C-family receptors [101]. GR has pleiotropic effects dependent on the cell type it is activated in, and regulates a broad spectrum of genes controlling development, metabolism, and the inflammatory response [101]. Two alternatively spliced isoforms of GR have been identified: the classic GR-alpha isoform responsible for mediating most known activity of GCs and the focus of study in this work, and the GR-beta isoform whose slightly altered LBD severely impedes its ability to bind GCs [101]. Several mechanisms for fine-tuning GR responses have been identified beyond ligand control, including post-translational modifications, variable cofactor recruitment, and an additional AF-2 region for interacting with cofactors in the LBD [101]. GR has been identified as a pioneer factor, capable of interacting with the SWI/SNF complex to remodel the global chromatin landscape in cells [102].

1.5 THERAPEUTIC RESISTANCE IN PROSTATE CANCER

The clinical benefits of ADT revolutionized the treatment of advanced PC [11]. However, these treatments are still considered palliative, as tumors develop a variety of strategies to evade ARSI. Roughly 10% of patients with advanced PC present with de novo resistance to first line ADT [41]. These tumors tend to be more androgen-independent, and a subset are characterized by their lineage plasticity towards a distinct neuroendocrine phenotype [103]. Neuroendocrine prostate cancer (NEPC) characteristically lacks normal markers of the luminal prostate, bear genetic aberrations to the tumor suppressors p53 and RB, and contain amplification of the MYCN oncogene [103]. While only 1% of primary PCs exhibit a NEPC characteristics when diagnosed by biopsy, the neuroendocrine phenotype is observed in nearly a quarter of mCRPC [104]. Interestingly, the PC specific TMPRSS2-ERG fusion gene is detected in NEPC roughly the same rate as overall PC, suggesting NEPC is still clonally derived from canonical PC [103]. Moreover,

these observations suggest that lineage plasticity to a neuroendocrine phenotype is likely a conserved mechanism for tumors to acquire resistance to ARSI. The precise molecular mechanisms facilitating this transition remain poorly understood and continue to be the subject of intense investigation.

The majority of mechanisms of resistance to ARSI can be characterized by a singular defining feature, the restoration of canonical AR signaling [105]. For most of the 20th century, AR was generally considered dispensable in PC; CRPC was thought to naturally exist in an androgen-independent state and therapeutic strategies were implemented accordingly [105]. In 1983, the LNCaP model of human prostatic carcinoma was established for laboratory research purposes [106]. Characterization of AR in this cell line exposed a T878A point mutation in the AR LBD [107]. Further studies in LNCaP cells revealed this mutation allowed other steroid hormones to promiscuously bind AR and drive canonical AR signaling and growth [108]. In 1993, this mutation was detected clinically in mPC, but not patient-matched primary samples, suggesting tumors developed an adaptive mutation to confer anti-androgen resistance [109]. Additional evidence of an essential role for AR in CRPC growth was reported in 1995, when genomic amplification of AR was observed in 30% patients with CRPC but not in patient-matched tumor samples prior to first line ADT [110]. Transcriptomic analysis revealed elevated levels of AR and associated cofactors were sufficient to restore androgen signaling to normal despite castrate-level ligand [111]. In isogenic xenograft models, elevated AR mRNA was the only notable difference between castrate-sensitive and castrate-resistant cells [112].

By the turn of the century, the importance of AR signaling in CRPC had been definitively established, unleashing a wave of investigation to identify novel mechanisms of resistance. Several next-generation sequencing studies examining the mutational landscape in PC have found multiple

recurring point mutations in the AR LBD associated with CRPC, notably A878T, F877L, W742C, and L720H [33, 113, 114]. Mutations to these residues expand the catalog of ligands able to bind AR, and cause antiandrogens to undergo an antagonist-to-agonist switch that drives tumor progression [115, 116]. While relatively rare, recurring mutations in other domains of AR have been observed [86]. D221H and G142V point mutations in the AR NTD sensitize AR to castrate-levels of ligand [117]. The E255K mutation prevents AR degradation by the proteasome causing increased levels of AR protein expression [118]. Within the DBD, the T575A mutation promotes non-specific DNA binding and alters the AR cistrome [119]. Recurring AR mutations have been used as predictive markers of disease and therapeutic response, as well as novel targets for therapeutic design [105].

Intratumoral androgen biosynthesis can also contribute towards therapeutic resistance. While castration reduces the concentration of systemic androgens up to 90%, the concentration of localized intraprostatic androgens in CRPC can remain at pre-castrate levels [120]. Intratumoral steroidogenesis can be mediated by the conversion of adrenal androgens to DHT, with CRPC tumors frequently displaying elevated levels of critical enzymes for steroid biosynthesis [121]. Alternatively, this can be achieved through a de novo androgen biosynthesis backdoor synthetic pathway [122]. Importantly, CYP17A1 is involved in each androgen biosynthesis pathway. The identification of intratumoral androgen synthesis as a driver of CRPC progression was the catalyst for developing CYP17A1 inhibitors to treat PC, culminating in the success of abi [123].

Not all mechanisms of resistance that restore androgen signaling require androgens. CRPC is frequently characterized by the presence of constitutively active AR splice variants, exemplified by AR-V7 [94]. Several pre-clinical studies of AR-V7 demonstrate its abilities to promote prostate growth *in vitro* and *in vivo* [98, 124, 125, 126, 127]. Further experiments found

that AR-V7 confers resistance to enz and can bring AR into the nucleus to bind chromatin in the absence of ligand [125]. While AR-V7 is able to regulate several canonical AR genes, it also regulates a unique set of genes particularly enriched for cell cycle functions [128]. AR-V7 additionally represses several tumor suppressors whose absence contributes to tumor progression [129]. The AR-V7 cistrome is governed by HoxB13, which collaborates with AR-V7 to upregulate target oncogenes [130,131]. AR-V7 can bind DNA as a homodimer or as a heterodimer with AR-FL, and unique binding sites have been identified dependent on the manner of dimerization [125,129,130,131,132,133]. The lack of an LBD inherently renders AR-V7 unaffected by all current FDA-approved antiandrogen therapies [94]. Several studies have demonstrated the clinical value of AR-V7. The 2014 PROPHECY trial, investigating the relationship between AR-V7 and current antiandrogen therapies, found detection of AR-V7 mRNA in circulating tumor cells (CTCs) predicted a lack of response to abi and enz [134]. It was further concluded that higher levels of AR-V7 CTC mRNA correlate with shorter times to treatment failure [135]. These studies established AR-V7 as a key clinical biomarker to predict responses and inform treatments that optimize patient responses [94].

Another androgen-independent mechanism of resistance to ARSI is mediated by the GR. The role of GR in PC is complex, and dependent on the activation state of AR [136]. When ligand-induced AR signaling is active, GR expression is repressed, and activation of GR with GCs has a growth-inhibitory effect [137,138]. Evidence of an alternative role for GR in PC was first described by Russell Szmulewitz, who observed elevated levels of GR in tumors following androgen deprivation [139]. Szmulewitz and others later reported a specific mechanism through which GR is reversibly upregulated following ARSI [140,141]. GR shares high homology with AR, particularly within the DBD, and is able to bypass AR to recognize and bind shared AR/GR

elements in the genome [140,141]. GR regulates several canonical AR target genes, as well as unique genes, that contribute to maintenance of the resistant phenotype [140,141,142]. Treatment with the GR antagonist mifepristone inhibited CRPC growth in pre-clinical models [143,144]. Clinically, there is significantly higher GR expression in CRPC compared to primary tumors, and elevated GR expression correlates with shorter progression free survival [145].

Mechanisms of resistance to ARSI have emerged as promising therapeutic targets for treating CRPC [105]. Early trials of GR antagonism with mifepristone in CRPC showed minimal efficacy, attributed to the high affinity of mifepristone for AR and PR as well as GR, and its weak AR agonist activity [146]. This failure necessitated the development of more specific GR antagonists without cross reactivity known as selective glucocorticoid receptor modulators (SGRMs) [146]. These non-steroidal highly specific ligands demonstrate potent inhibition of GR signaling in the prostate and inhibit CRPC progression in preclinical models [147]. Notably, SGRMs demonstrated stronger efficacy in xenograft models expressing higher levels of GR [147]. The success of SGRMs in preclinical models has resulted in two SGRMs, Relacorilant and CORT125281, progressing into clinical trials in patients (NCT03674814, NCT03437941).

CHAPTER 2

MATERIALS AND METHODS

2.1 Cell Lines and Reagents

2.1.1 Cell Lines and Media

The 22RV1 and HEK293T cell lines were generous gifts from Dr. John Isaacs (Johns Hopkins University) and Dr. Donald Vander Griend (University of Illinois Chicago) respectively. The VCaP cell line was purchased from the American Type Tissue Culture (ATCC). All cell lines were routinely authenticated using short tandem repeat profiling at the University of Arizona and tested for mycoplasma contamination using the ATCC Universal Mycoplasma Detection Kit (ATCC). 22RV1 cells were grown in RPMI-1640 (Gibco) supplemented with 2mM L-glutamine, 1% Penicillin/Streptomycin (Corning) and 10% FBS (Atlanta Biologicals). VCaP and HEK293 cells were grown in DMEM (Gibco) supplemented with 2mM L-glutamine, 1% Penicillin/Streptomycin (Corning) and 10% FBS (ATCC).

2.1.2 Derivative Cell Lines

In order to control AR-V7 expression, a lentiviral system was utilized to deliver shRNA into cells for stable integration in the genome and inducible expression with doxycycline treatment. HEK293T cells were plated in normal media for 24 hours then treated with transfection media (8mL Optimem, 54µL Mirus TransIT-2020 reagent, 15µg pLKO shAR-V7 or shCtrl plasmid, 10µg pspax2 plasmid, 10µg apmd2.g plasmid) overnight. After 24 hours, transfection media was removed, and full media was added to each plate. After 72 hours media containing lentivirus is removed, filtered, centrifuged, and resuspended in standard media for the cell type destined for infection. Parental cells are plated and treated with virus-containing media with 5µg/mL

polybrene. Virus-containing media is changed every 24 hours for 3 days with fresh polybrene added each time. After three days viral media is removed, cells are washed, and antibiotic selection is initiated. The following cell line derivatives were generated: 22RV1 shV7, 22RV1 shCtrl, 22RV1 nGFP, 22RV1 shV7 nGFP, 22RV1 shCtrl GFP, VCaP shV7, VCaP shCtrl.

2.1.3 Cell Culture

22RV1 cells were passaged in standard media upon 80% confluency at a 1:5 dilution. VCaP cells were passaged in standard media upon 80% confluency at a 1:3 dilution. HEK293T cells were passaged in standard media upon 80% confluency at a 1:10 dilution. Derivative cell lines were maintained using the same strategies as their parental cell lines, with the notable exception of undergoing antibiotic selection every other passage. Induction of shRNA in derivative cell lines was achieved by 96-hour pretreatment with media containing 3μg/mL doxycycline before initial plating. For experiments, cells were plated in standard media and incubated overnight at 37°C. After 24 hours, cells were washed with PBS (Hyclone), and placed in media containing 10% charcoal stripped FBS (Atlanta Biologicals). The following compounds were added to media as specified: 3.5nM R1881 (Sigma Aldrich), 10μM enzalutamide (Selleck Chemicals), 100nM dexamethasone (MP Biomedicals), 10μM CORT125134 (Corcept Therapeutics). Cells were treated for three days with R1881 and enzalutamide, changing the media after 48 hours. After 72 hours dexamethasone and CORT125134 were added for six hours (RNA) or 1 hour (all other experiments). Doxycycline treatment at 3μg/mL was maintained throughout the course of experiments that involved shRNA knockdown, but not during normal cell passaging.

2.2 Protein Analyses

2.2.1 Western Blotting

Protein lysates were loaded onto 8% SDS-polyacrylamide gels, resolved for 1 hour at 100V, and transferred to nitrocellulose membranes overnight at 22V (LI-COR). Membranes were blocked in 5% dry milk, incubated with indicated primary antibody overnight in 5% BSA in TBS, then washed three times with TBST. Fluorescently labeled secondary antibodies were incubated for 2 hours in 5% dry milk in TBST, then washed three times with TBST. Proteins were detected with an Odyssey imaging system (LI-COR).

2.2.2 NanoBRET Assays

Plasmids for our hormone receptor fusion proteins were generously provided by Dr. Geoffrey Greene (University of Chicago). HEK293 cells were plated in Greiner CELLSTAR® 96-well plates (Sigma Aldrich) and incubated overnight at 37°C. After 48 hours, 10μL of transfection mix (1 mL Optimem media, 10μg halo-tag plasmid, 1μg NanoLuc luciferase plasmid, 25 μL Mirus TransIT-2020 reagent) was added to each well and left to incubate overnight. After 72 hours, nuclear hormone receptor ligands were added at specified concentrations and left to incubate overnight. After 96 hours, halo-tag 618 ligand (Promega) was added to each well and left to incubate for 3 hours. Finally, Nano-Glo® substrate (Promega) was added to each well immediately before imaging plates with a Synergy Neo2 Multi-Mode Plate reader at 450nm and 610nm.

2.2.3 Co-Immunoprecipitation Assays

Nuclear fractionation was performed using the NE-PER kit (Thermo Fisher Scientific). Cells were lysed manually with a 28.5-gauge syringe. Protease and phosphatase inhibitors (Roche) were added fresh before lysis. Lysates were cleared with 50μL of a 50:50 slurry of Protein A and Protein G Dynabeads (Thermo Fisher Scientific) and 1μg of normal rabbit IgG (Cell Signaling

Technology) for one hour rotating at 4°C. After removal of the Dynabeads, lysates were incubated with another 50μL of a 50:50 slurry of Protein A and Protein G Dynabeads and 2μg primary antibody and rotated overnight at 4°C. GR was pulled down using a rabbit anti-GR mAb (D8H2, Cell Signaling Technology). Normal rabbit IgG (Cell Signaling Technology) was used as a negative control. Proteins were eluted from the beads using Laemmli buffer and visualized using western blotting.

2.3 RNA Analyses

2.3.1 RNA Harvest

RNA was recovered using the RNAeasy Mini Kit with optional DNAse digestion (Qiagen) according to the manufacturers protocol. The concentration of harvested RNA was measured with a NanoDrop spectrophotometer (Thermo Fisher Scientific). RIN scores were determined by the University of Chicago Functional Genomics core using an Agilent Bioanalyzer 2100 (Agilent Technologies).

2.3.2 qRT-PCR

Purified RNA was converted to cDNA by reverse transcription using the SuperScript III Reverse Transcriptase kit (Invitrogen) according to the manufacturers protocol. Gene expression was quantified using Power SYBR Green Master mix (Invitrogen) with custom primers according to the manufacturers protocol. PCR amplification of cDNA was done with a LightCycler (Roche Applied Science). Standard curves were used to assess primer efficiency. The average change in threshold cycle (ΔCT) was determined for each of the samples relative to endogenous GAPDH levels and compared to vehicle control (ΔΔCT). All experiments were performed in triplicate

2.3.3 RNA-Seq

Samples with a RIN score ≥9 were selected for library preparation at the University of Chicago Functional Genomics core. RNA-sequencing libraries were prepared from 2µg purified RNA with the KAPA-Stranded mRNA-Seq Kit (KAPA Biosystems), using oligo-dT magnetic beads to enrich for mRNA. Fragment size of sample libraries was measured using a 2200 TapeStation system (Agilent). Library adapters 1-12 (KAPA Biosystems) were used to barcode samples and multiplex up to six samples per lane. Sequencing was performed on a NovaSeq machine (Illumina) in 100bp, paired-end runs. Sequencing data was collected over multiple flow cells and returned in FASTQ format.

2.3.4 RNA-Seq Analysis

All RNA-Seq reads were analyzed using the FastQC tool suite to ensure quality sequencing. Low quality and adapter sequences were trimmed using trimmomatic. Trimmed reads were aligned to the GRCh38/hg19 reference genome using STAR. The SamTools suite was used to convert files to BAM format and remove duplicate reads. Differentially expressed genes were determined with DESeq2 and filtered by fold change ≥1.5. Ingenuity pathway analysis (Ingenuity systems) of differentially expressed genes (DEGs) was performed to identify functional pathways that are significantly altered under particular conditions.

2.4 DNA Analyses

2.4.1 Plasmid Stocks

Several plasmids were used for this work and are maintained in glycerol stock solutions for long term storage and future use. Plasmids received in bacterial stocks were grown in LB media and isolated with a Plasmid Miniprep Kit (Qiagen). Plasmids received on filter paper were resuspended in TE buffer. Isolated plasmids were sent to the University of Chicago Sequencing

core for sequence validation. Plasmids with confirmed sequences were transformed into competent cells and underwent antibiotic selection. Competent cells containing verified plasmids selected with antibody were then combined 1:1 with glycerol for long term storage at -80°C.

2.4.2 ChIP-PCR

ChIP was performed using the iDeal ChIP-seq kit for Transcription Factors (Diagenode) according to the manufacturer's protocol. Generally, cells were fixed with 1% paraformaldehyde and sonicated with a Bioruptor Pico (Diagenode). Sonicated DNA fragment size and concentration were determined with an Agilent Bioanalyzer 2100 (Agilent Technologies). Proteins of interest were immunoprecipitated using primary antibodies conjugated to Protein A beads. Species matched normal IgG antibodies were used as a control. Eluted ChIP DNA was purified and recovered using the PCR purification Kit (Qiagen). Equal volumes of eluted ChIP DNA were used in place of cDNA for standard qPCR described above to determine chromatin occupancy at specific genomic sites.

2.4.3 ChIP-re-ChIP

Our protocol for ChIP-re-ChIP follows a previously published methodology (furlan-magaril). Cross-linking, sonication, and immunoprecipitation are carried out in the same manner as a normal ChIP experiment. Following the first immunoprecipitation, all material pulled down is resuspended and undergoes a second round of immunoprecipitation with a different antibody. Eluted ChIP-re-ChIP DNA is purified and recovered using the PCR purification Kit (Qiagen) then analyzed using qPCR to determine whether two proteins of interest co-occupy particular genomic sequences.

2.4.4 ChIP-Seq Analysis

Purified ChIP-DNA was sent to the University of Chicago Functional Genomics core for library preparation and sequencing. ChIP-seq libraries were prepared using the KAPA LTP Library Preparation Kit (KAPA Biosystems) and sequenced on a NovaSeq machine (Illumina) in 100bp, paired-end runs. All ChIP-seq reads were analyzed using the FastQC tool suite to ensure quality sequencing. Low quality and adapter sequences were trimmed using trimmomatic. Trimmed reads were aligned to the GRCh38/hg19 reference genome using Bowtie2. The SamTools suite was used to convert files to BAM format and remove duplicate reads. MACS2 was used for peak calling using the narrowPeaks method. BED files were used to identify and annotate peaks, and peaks were visualized using the Integrative Genomics Viewer (Broad Institiute) following conversion to BigWig format.

2.5 Immunofluorescent Assays

2.5.1 Cell Fixation and Permeabilization

22RV1 cells and VCaP cells were seeded in full media in an 8-well glass chamber slide (Corning) and incubated at 37°C overnight. After 24 hours, the media was aspirated, and cells began treatment as described previously. After treatment, cells were fixed with 4% paraformaldehyde in PBS for 20 minutes then quenched with 50mM NH_4Cl for 10 minutes. Cells were then washed 3 times with PBS and once with MilliQ ddH_2O to remove any salts. Cells were permeabilized with 0.25% TritonX-100 in PBS for 20 minutes at room temperature and washed 3 times with PBS.

2.5.2 Immunofluorescent Staining

Fixed and permeabilized cells were blocked with 10% normal donkey serum (Sigma) in PBS for one hour at room temperature then incubated with primary antibodies diluted in blocking

buffer for 2 hours at room temperature. Cells were then washed 3 times with PBS and incubated with secondary antibodies (Jackson ImmunoResearch, Westgrove PA) diluted in blocking buffer for 1 hour at room temperature. Cells were washed, counterstained with Hoechst 33342 (Thermo Fisher Scientific) and mounted with ProLong Gold Antifade (Invitrogen).

2.5.3 Proximity Ligation Assays

Proximity ligation assays rely on the Duolink In Situ Red Starter Kit Mouse/Rabbit (Sigma Aldrich) generally following the manufacturers protocols. All incubations occur in a humidity chamber. Fixed and permeabilized cells are blocked for 30 minutes in a 37°C. Blocking solution is removed and primary antibodies are added and left to incubate overnight at 4°C. Cells are washed and incubated with secondary PLA probes for 1 hour at 37°C. Cells are then washed again and incubated with ligase for 30 minutes at 37°C. Cells are once again washed then incubated with polymerase for 100 minutes at 37°C. Following a final wash, slides were mounted with DuoLink In Situ Mounting Medium with DAPI.

2.5.4 Microscopy and Image Analysis

Imaging of slides was done at the University of Chicago Microscopy core with a Marianas Yokogawa type spinning disk inverted confocal fluorescence microscope (Slidebook, version 6) using 40x-oil objective. All images were taken as 10-micron thick z-stacks with a 0.4-micron step size and max projections of each z-stack were used for image analysis. The number of PLA signals per nuclei was determined by setting a binary threshold, and using a water shed segmentation and counting algorithm on five representative images per condition. Each field was manually confirmed to verify the correct number and localization of PLA signals.

2.6 Proliferation Assays

2.6.1 Counting Assays

Cells were seeded and treatment was initiated after 24 hours. Media changes occurred every 48 hours. Cells were counted after 7-, 10-, and 14-day timepoints using a Vi-Cell machine (Beckman Coulter). All experiments were performed in triplicate.

2.6.2 Incucyte Assays

22RV1shV7 and 22RVshCtrl cells expressing nuclear GFP were pretreated as described above. Cells were seeded in Greiner CELLSTAR 96-well plates at a density of 10,000 cells per well and allowed to sit overnight. After 24 hours, ligands were added and the plate was immediately placed in an Incucyte S3 Live Cell Analysis System (Essen Bioscience). Images of cells were collected every 4 hours for one week. The media was changed once after 72 hours by removing one half volume from each well and adding one half volume with 2x concentration of ligands plus doxycycline. The number of cells per image was counted using built-in analysis software. All experiments were performed in triplicate.

2.7 Statistical Analysis

For experiments performed in triplicate, a one-way student's t-test was used determine significance between two conditions (qPCR, NanoBRET) and one-way ANOVA was used to determine significance between multiple conditions (qPCR, NanoBRET). Statistical comparisons between multiple groups with varying sample sizes were calculated using one-way ANOVA with post-hoc Tukey HSD test (PLA quantification). Proliferation experiments were also performed in triplicate, and Tukey's multiple comparisons test was used to determine the significance between groups over time.

2.8 Reagent Toolbox

2.8.1 Antibodies

Table 2.1 Antibodies used

Company	Target	Identifier	Application
RevMab Biosciences	AR-V7	RM-7	WB, IP, IF
Precision Antibody	AR-V7	AG10008	WB, IP, IF
Cell Signaling Technology	GR	D8H2	WB, IP, IF
BD Biosciences	GR	Clone 41	WB, IF
Santa Cruz biotechnology	GR	G-5	IP
Abcam	GR	BuGR2	IP
Abcam	AR-FL	EP670Y	WB
Sigma Aldrich	actin	AC-74	WB
Cell Signaling Technology	laminB1	D4Q4Z	WB
Cell Signaling Technology	alpha-tubulin	2144	WB
Cell Signaling Technology	Normal IgG (r)	2729	IP
Cell Signaling Technology	Normal IgG2a (m)	E5Y6Q	IP

2.8.2 Primers

Table 2.2 Primers used for PCR

Gene	Orientation	Target	Primer
FKBP5	Forward	promoter	5'-ACCTCCTCACGTGTTCTTGG-3'
FKBP5	Reverse	promoter	5'-AACATTTTGTCCGTTCCGCA-3'
KLK3	Forward	promoter	5'-TGCTCAGCCTTTGTCTCTGA-3'
KLK3	Reverse	promoter	5'-CCTCCAGAGTAGGTCTGTTTTCA-3'
AR-V7	Forward	cryptic exon 3	5'-CTACTCCGGACCTTACGGGGACATGCG-3'
AR-V7	Reverse	cryptic exon 3	5'-TGCCAACCCGGAATTTTTCTCCC-3'
GAPDH	Forward	exon 1	5'-GAGTCAAGGATTTGGTCGT-3'
GAPDH	Reverse	exon 1	5'-TTGATTTTGGAGGGATCTCG-3'

CHAPTER 3

CHARACTERIZATION OF A NOVEL ENDOGENOUS INTERACTION BETWEEN GR AND AR-V7

3.1 Introduction

Acquired resistance to ARSI remains the major clinical challenge for the treatment of advanced PC. Consequently, understanding these mechanisms of resistance has been the subject of intense investigation throughout the 21st century [105]. Several studies identified the splice variant AR-V7 as a driver of CRPC progression [94]. AR-V7 is expressed in between 75-90% of patients with mCRPC, and is associated with disease progression, therapeutic resistance, and poor clinical outcomes [33,94,95,134,148]. Following ARSI, AR-V7 is able to partially restore canonical androgen signaling pathways as well as drive unique gene expression that contributes to tumor progression [94,128,129].

GR has separately been identified as a driver of CRPC progression. Following ARSI, GR is upregulated and can bypass AR to bind shared AR/GR response elements in the genome and regulate compensatory signaling that promotes tumor proliferation [140,141]. Traditionally, GR and AR-V7 have been thought to drive resistance to ARSI independently [149]. However, they share several notable features. Both receptors have their expression upregulated following ARSI, both receptors partially restore canonical androgen signaling, and both receptors come from the NR3C-family of nuclear receptors [136]. Despite these similarities, no direct relationship between GR and AR-V7 has been investigated.

Our lab first detected evidence for a link between GR and AR-V7 when performing GR ChIP experiments. Immunoprecipitated fractions were isolated, and GR was visualized with

western blotting as a quality control measure. By chance, these blots were also probed with an N-terminal AR antibody revealing a striking breakthrough. AR-V7, but not AR-FL, was detected on the blot, indicating co-immunoprecipitation with GR. That simple observation became the catalyst for the subsequent work presented in this thesis. NR3C-family dimerization is mediated by the highly conserved D-box motif within the DBD [88]. However, NR3C-family receptors rely on specific contacts in the LBD to discriminate between different family members [90]. We hypothesized that because AR-V7 lacks an LBD but retains a functional DBD containing the D-box motif, AR-V7 could promiscuously heterodimerize with other members of the NR3C-family.

3.2 Results

3.2.1 AR-V7 can heterodimerize with liganded NR3C-family nuclear hormone receptors in a canonical D-box dependent manner

In order to evaluate the ability of AR-V7 to heterodimerize with other NR3C-family receptors, we utilized NanoBRET technology to measure protein-protein interactions [15]. A NanoLuc luciferase donor was fused to the N-terminal of AR-V7 (NL-V7), and a halo-tag acceptor was fused to the N-terminal of each NR3C-family receptor (HT-AR, HT-GR, HT-MR, HT-PR). A halo tag-was also fused to the N-terminal of the NR3A-family estrogen receptor-alpha (HT-ER), whose divergent evolution displays less conservation within the D-box motif compared to the NR3C-family [151]. Expression of a halo-tag alone without a receptor was also used as a control (HT).

NL-V7 and a halo-tagged construct were co-transfected into HEK293 cells. and the NanoBRET signal was measured following the addition of ligands. R1881 (AR), dexamethasone (GR), progesterone (PR), aldosterone (MR), and estradiol (ER) were used at 100nM as ligands and each ligand was trialed with every receptor combination. As hypothesized, AR-V7 demonstrated

the ability to interact with liganded NR3C-family receptors. The strongest interaction was observed between AR-V7 and AR-FL, although whether that is due to favorable binding or an over-saturation of ligand at 100nM remains unresolved. AR-V7 interacted with GR following treatment with dexamethasone. Interestingly, dexamethasone acts as a weak agonist of PR and was able to partially stimulate an interaction with AR-V7. Importantly, no interactions were detected between AR-V7 and ER, even when liganded with estradiol, hinting that this interaction might be mediated by the conserved D-box motif within the NR3C-family.

To ensure the observed interactions were caused by canonical D-box mediated heterodimerization, and not the result of an overall increase in the nuclear levels of halo-tag, we generated an A596T S597T mutant of NL-V7 (AR-V7^{mut}) to compare with the wild-type NL-V7 fusion receptor (AR-V7^{WT}). This mutation has clinical significance and is associated with androgen insensitivity syndrome, as failure to dimerize prevents AR from binding to DNA and regulating gene expression [152]. Furthermore, this mutation has previously been demonstrated to abrogate AR-V7 homodimerization, as well as dimerization between AR-V7 and AR-FL [125,153]. As previously reported, we found that mutation to the AR-V7 D-box disrupts the interaction between AR-V7 and AR-FL. Remarkably, we also found that this mutation was sufficient to impair interactions between AR-V7 and GR, as well as interactions between AR-V7 and PR. Taken altogether, this data suggests AR-V7 can heterodimerize with liganded NR3C-family nuclear hormone receptors in a canonical D-box mediated fashion.

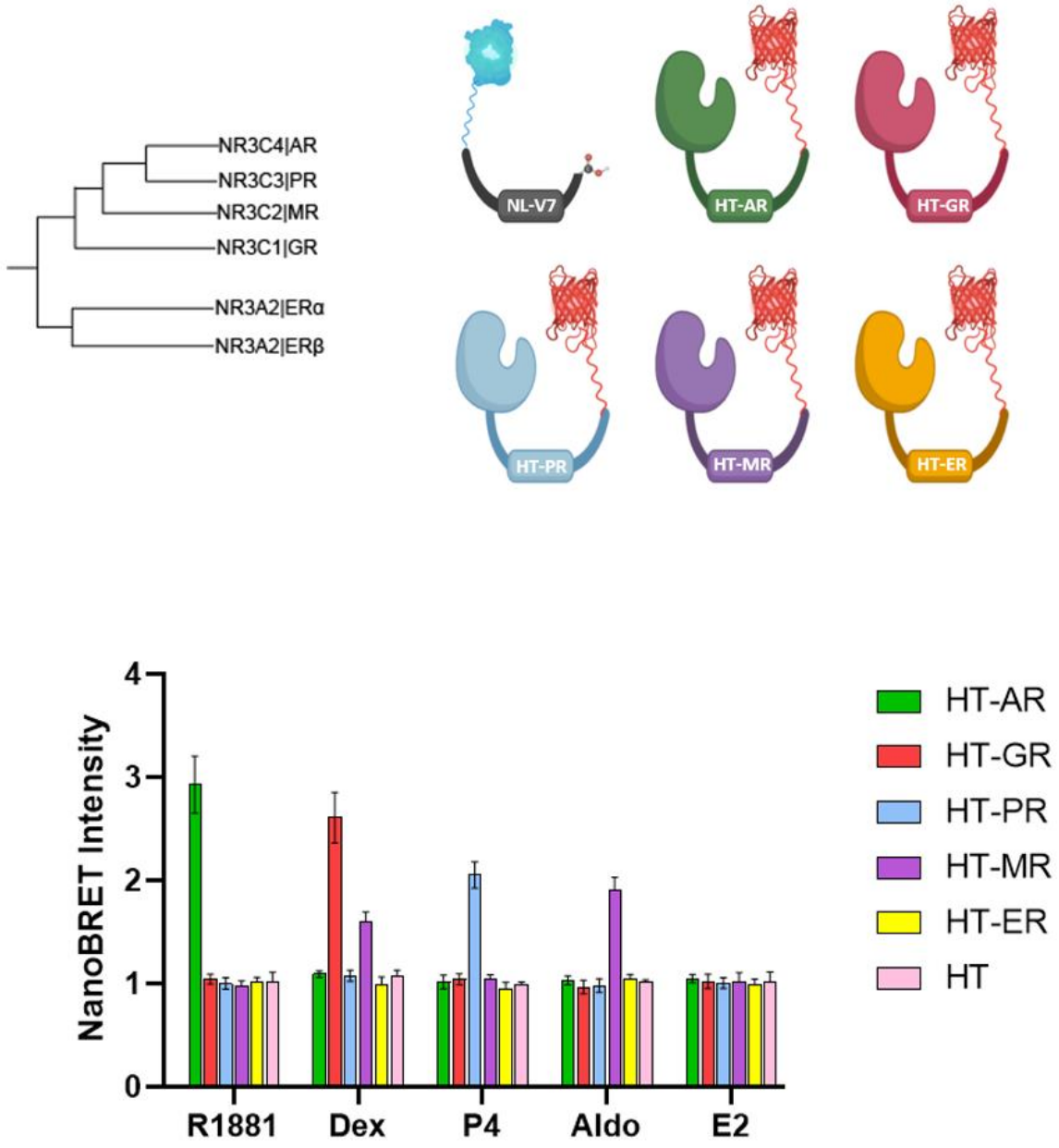

Figure 3.1 AR-V7 interacts with liganded NR3C-family receptors. All ligands were used at 100nM. No interaction was observed between AR-V7 and liganded NR3A-family ER suggesting this interaction is mediated by the conserved dimerization motif shared by NR3C-family receptors. Interactions with AR-V7 were detected when AR was liganded with R1881, GR was liganded with dexamethasone, PR was liganded with dexamethasone, and when MR was liganded with aldosterone or dexamethasone.

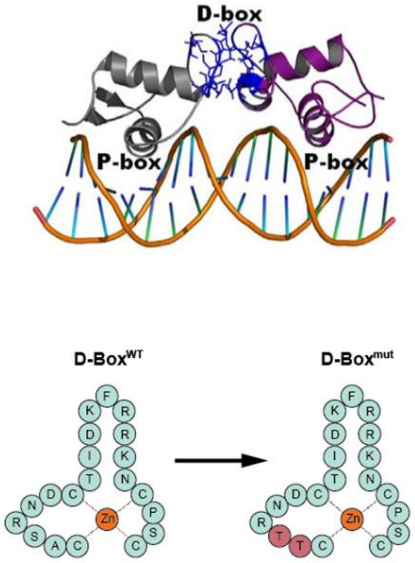

Figure 3.2 NR3C-family receptor dimerization is mediated by a highly conserved D-box motif within the LBD of each receptor. The A596T S597T mutation was introduced into the NL-V7 fusion protein to evaluate whether interactions between AR-V7 and liganded NR3C-family receptors occur through canonical D-box mediated interactions. Figure adapted from Dalal *et al.* [151].

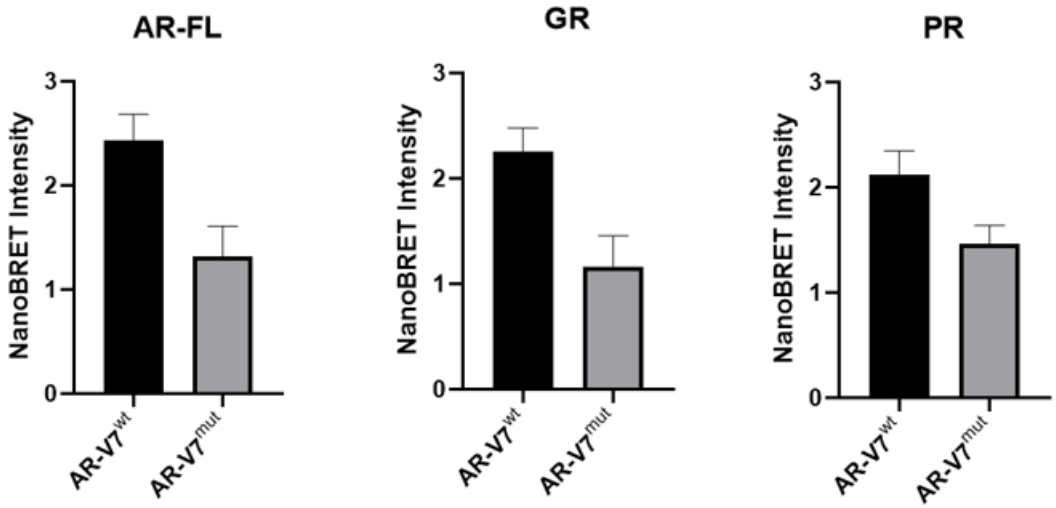

Figure 3.3 Mutation to the AR-V7 D-box significantly disrupted the interaction between AR-V7 and other NR3C-family receptors, indicating these interactions occur through canonical NR3C-family heterodimerization mediated by the D-box motif.

3.2.2 Endogenous AR-V7 heterodimerizes with GR following ARSI in prostate cancer cell lines and together they can co-occupy chromatin

While our NanoBRET assays demonstrate ectopically expressed AR-V7 heterodimerizes with liganded NR3C-family nuclear hormone receptors in HEK293 cells, this artificial system reveals little about any interactions between endogenous AR-V7 and GR in PC. To interrogate potential endogenous interactions between the two receptors we utilized VCaP and 22RV1, two PC cell lines that express GR and AR-V7 [154,155]. In the VCaP cell line, baseline GR and AR-V7 expression are low, similar to what is observed in patients, and the expression of both is induced following ARSI [155]. Alternatively, the 22RV1 cell line bears unique genomic and epigenomic features which allow for constitutive expression of AR-V7 and GR [156,157]. Immunofluorescent staining revealed co-expression of nuclear GR and AR-V7 at the single-cell level in each cell line following ARSI.

In order to assess whether AR-V7 and GR interact in PC, we performed nuclear fractionated co-immunoprecipitation assays in VCaP and 22RV1 cells. Interestingly, an interaction between GR and AR-V7 was detected, but only following ARSI. Furthermore, we found that this interaction occurs within the nucleus, and appears to be strongest in conditions when GR is liganded, consistent with the interactions observed in our NanoBRET assays.

To better quantify the endogenous interactions between AR-V7 and GR in our PC cell lines we performed Duolink proximity ligation assays. In each cell line, when androgen signaling is intact, there is no detectable interaction by PLA, similar to CoIP. Following ARSI, which restricts AR-FL ligand-dependent dimerization, a nuclear interaction between GR and AR-V7 is observed. Furthermore, when GR is liganded and activated, the number of nuclear interactions between AR-V7 and GR detected by PLA increases significantly ($p < 0.01$).

Taking this into account, we next hypothesized that if GR and AR-V7 were heterodimerizing in PC cell lines subsequent to ARSI and GR activation, then they would be able to co-occupy DNA to initiate transcription. To test this, we performed ChIP-PCR at the promoter of the canonical AR/GR target gene FKBP5, where both GR and AR-V7 occupancy was detected. Sequential ChIP (ChIP-re-ChIP) of GR then AR-V7 followed by PCR also revealed enrichment at the FKBP5 promoter, suggesting GR and AR-V7 are able to co-occupy this site following ARSI when GR is liganded. Taken altogether, this data suggests AR-V7 can utilize GR as an alternative partner for heterodimerization that binds chromatin in PC following ARSI.

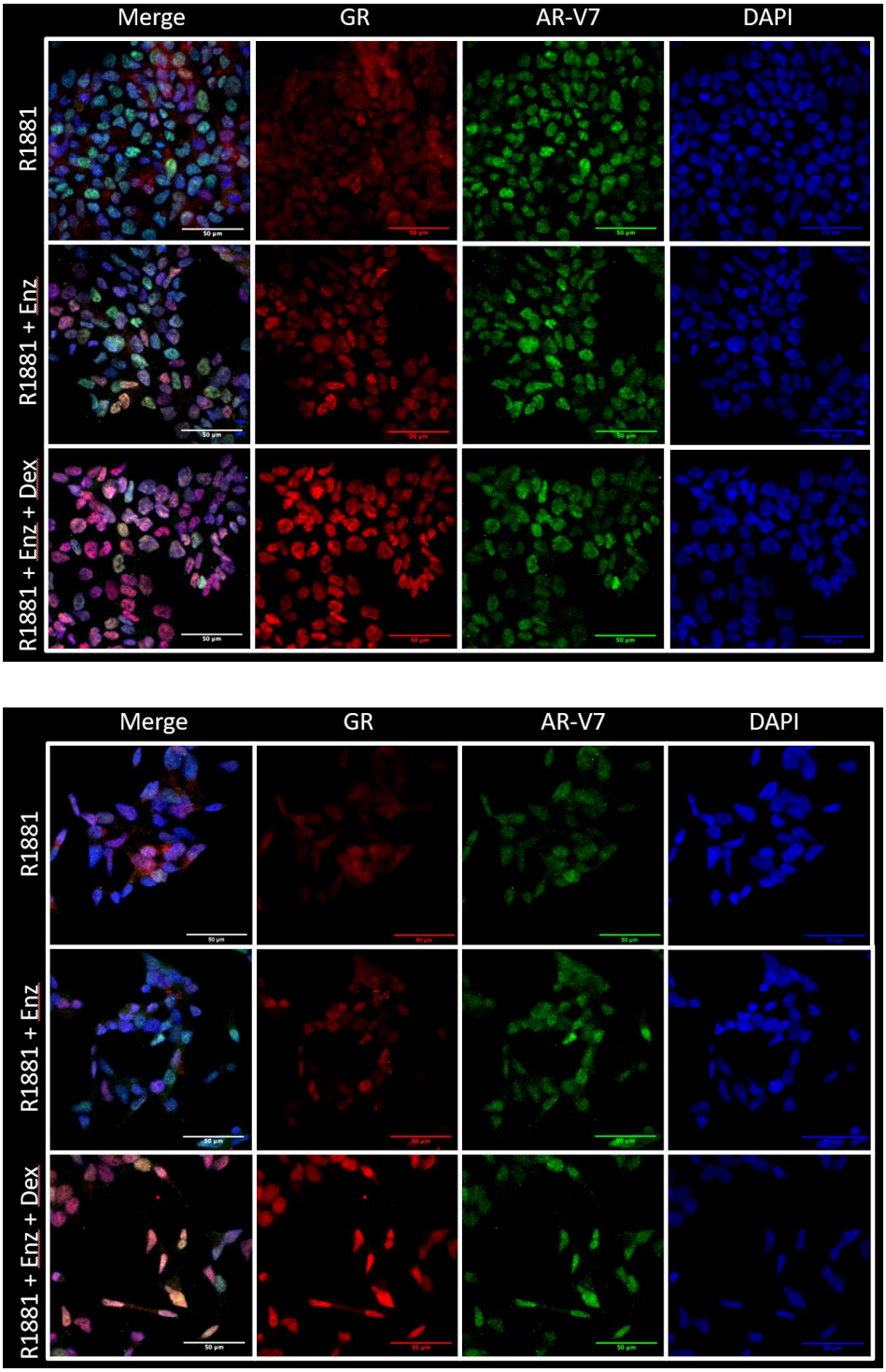

Figure 3.4 Endogenous expression of GR and AR-V7 in 22RV1 (top) and VCaP (bottom) cells. GR and AR-V7 expression is induced following ARSI in VCaP cells, whereas 22RV1 cells displays constitutive expression of each receptor. Following ARSI, dexamethasone drives GR nuclear localization in both cell lines.

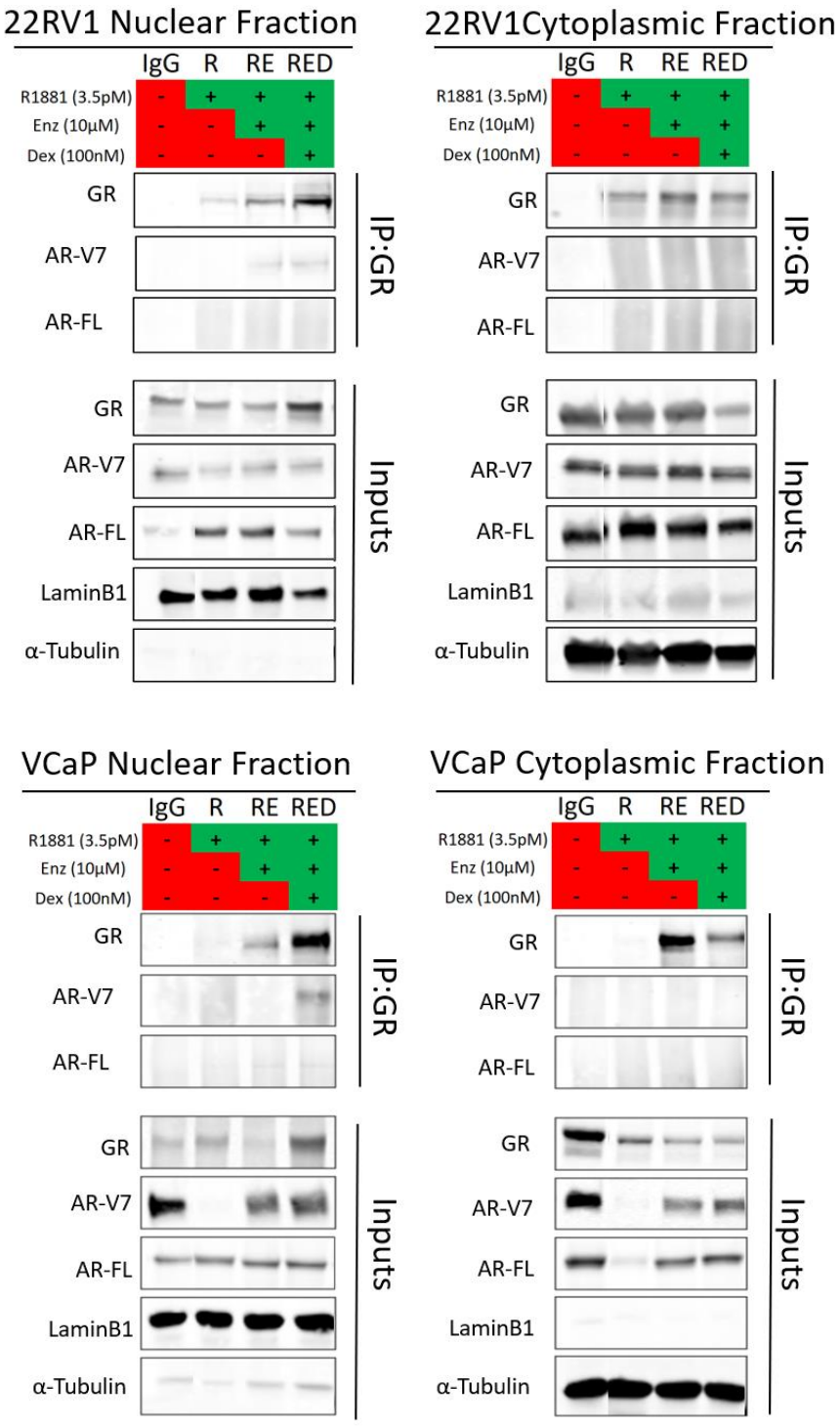

Figure 3.5 Co-immunoprecipitation assays following nuclear fractionation reveal a novel endogenous interaction between GR and AR-V7 in prostate cancer. This interaction is exclusively detected within the nucleus, only observed subsequent to ARSI, and strongest when GR is liganded with dexamethasone.

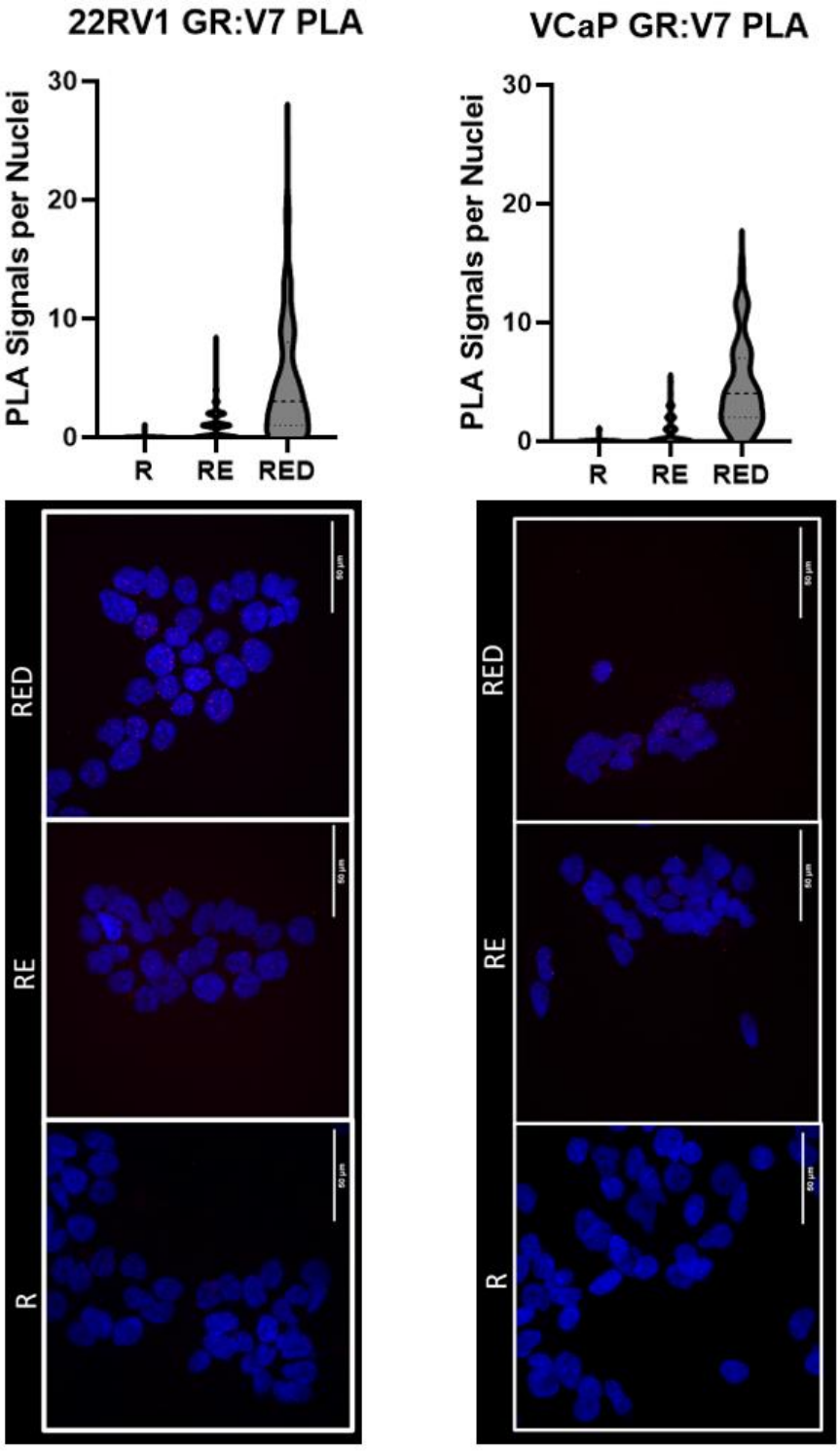

Figure 3.6 PLA reveals a novel endogenous interaction between GR and AR-V7 in prostate cancer. The interaction is only observed following ARSI, and significantly more interactions are detected when GR is liganded with dexamethasone.

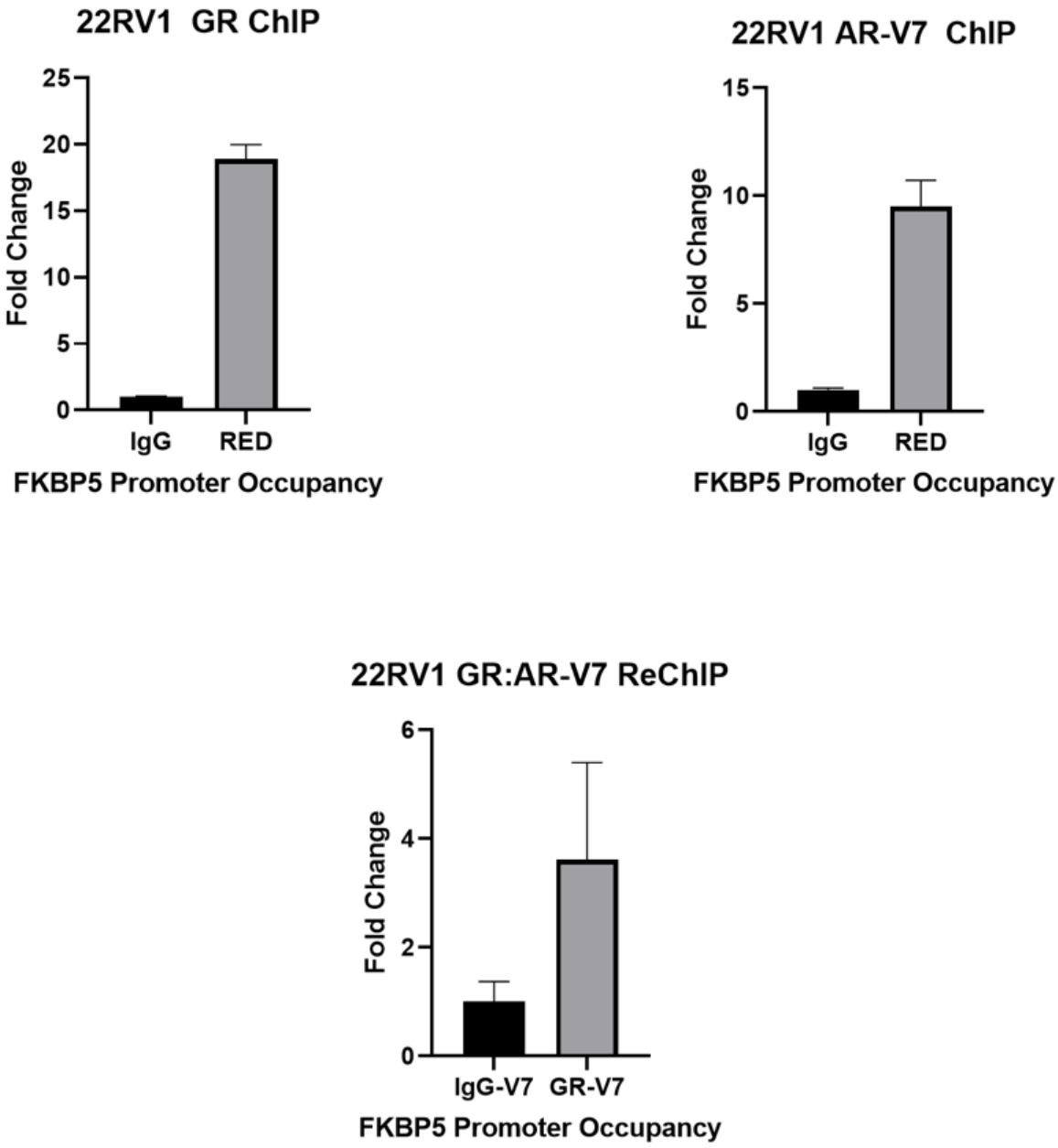

Figure 3.7 GR and AR-V7 co-occupy the promoter of the canonical AR target gene FKBP5 (bottom). GR occupancy (top left) and AR-V7 occupancy (top right) were determined by ChIP-PCR. Co-occupancy of both receptors at this site was detected by ChIP-re-ChIP-PCR.

3.3 Discussion

Dimerization has long been established as a requirement for AR-V7 transcriptional activation [100,125,153]. However, whether that occurs primarily through homodimerization or heterodimerization has been a subject of debate for scholars in the field. AR-V7 activity was originally thought to be dependent on AR-FL, and thus a model of heterodimerization was initially proposed [158]. This view was directly challenged when AR-V7 alone was demonstrated as sufficient to drive growth in cells lacking AR-FL [100]. Subsequent research determined that both heterodimerization and homodimerization occurs at the chromatin level, and that a fraction of AR-V7 binding sites are uniquely occupied by either homodimers or heterodimers [100,125,129,130,153]. AR-V7 and AR-FL co-occupy the promoters of canonical AR target genes in a mutually dependent manner, suggesting that AR-V7 dimerization is partially determined by the partners available for it to bind under given physiological conditions [125]. Recent studies have suggested AR-V7 has a preference for homodimerization following ARSI [130]. However, homodimerization in this context was only characterized by the absence of AR-FL, and not by the present of two AR-V7 proteins.

Here, we have identified several novel binding partners for AR-V7 within the NR3C-family of receptors. Furthermore, we have verified that these interactions occur through canonical NR3C-family heterodimerization mediated by the D-box motif. Elevated expression of AR, GR, and PR has previously been reported in CRPC compared to primary tumors and is associated with worse clinical responses [1,41,105,147,159]. Indeed, AR-V7 heterodimerization with AR-FL has been directly linked to driving oncogenic signaling [129,130,133]. However, the functional consequences of AR-V7 heterodimerization with other NR3C-family receptors have not been

explored. Our lab has published extensively on the role of GR in PC, and we chose to focus our analysis within the prostate on that interaction specifically [139,140,142,147].

We used multiple methodologies to conclude that AR-V7 can utilize GR as an alternative partner for heterodimerization in PC. In each experiment, the novel endogenous interaction between AR-V7 and GR was detected primarily in the nucleus and was only observed subsequent to ARSI. Notably, there were significantly more interactions observed between GR and AR-V7 when GR was liganded with dexamethasone. These observations further support a model for AR-V7 dimerization dictated by the availability of dimerization partners under given physiological conditions. In CRPC, AR-FL nuclear translocation and DNA-binding is restricted, while GR and AR-V7 have their expression upregulated and can localize to the nucleus unimpeded, creating conditions favorable for GR to be a preferred binding partner for AR-V7.

The ability for AR-V7 and GR to heterodimerize and co-occupy the promoter of a canonical AR target gene fundamentally transforms the current understanding of hormone receptor biology. Future studies into AR-V7 must reconsider the methodology for determining AR-V7 homodimerization, with these results demonstrating that the absence of AR-FL is an insufficient metric. Furthermore, these results call into question past characterizations of the AR-V7 cistrome. Robust investigation to fully characterize the AR-V7 dimerization landscape in PC should be initiated to fully understand the role this receptor plays in driving oncogenic signaling.

CHAPTER 4

AR-V7 AND GR COORDINATE TO DRIVE ONCOGENIC PHENOTYPES IN PROSTATE CANCER FOLLOWING ARSI

4.1 Introduction

Maintained androgen signaling is essential for most PC growth and is a hallmark of resistance to therapy [105]. Because GR and AR-V7 share many similarities with AR-FL, particularly within the DBD, these receptors are able to recognize, bind, and regulate the expression of many AR target genes [140,141]. However, the unique biology of each receptor also allows for the regulation of distinct genes that contribute to tumor progression. Gene expression regulated by AR-V7 independent of AR-FL is enriched for cell-cycle genes and DNA-damage repair genes [100,129]. Alternatively, unique gene expression driven by GR in PC is associated with lipid metabolism, cAMP activation, and nucleic acid metabolism [142].

This report of a novel endogenous interaction between GR and AR-V7 is the first evidence for potential cooperation between two receptors historically thought to drive CRPC progression independently. Both AR-FL and GR have demonstrated an ability to heterodimerize with AR-V7 to co-occupy the promoter of canonical AR target genes. However, investigations into the functional consequences of AR-V7 heterodimerization to date have been limited to AR-FL. These studies have revealed distinct sites where AR-V7 and AR-FL co-occupy chromatin and regulate oncogenic signaling [125,133,153]. Accordingly, AR-FL homodimerization, AR-V7 homodimerization, and AR heterodimerization each regulate unique signaling pathways that contribute to tumor progression [133,153]. We hypothesized that a similar phenomenon could

occur with GR, where coordinated activity between GR and AR-V7 drives unique gene expression accelerating tumor progression.

4.2 Results

4.2.1 Coordinated activity between GR and AR-V7 drives unique transcriptional profiles following ARSI

Parental cell lines were genetically modified to control the expression of AR-V7. A lentiviral system was used to deliver a doxycycline inducible shRNA either targeting the AR-V7 transcript or a scramble control, generating four derivative cell lines: 22RV1 shV7, 22RV1 shCtrl, VCaP shV7, and VCaP shCtrl. GR activity was controlled by treating cells with or without dexamethasone. To identify genes regulated by coordinated activity between GR and AR-V7 we performed RNA sequencing for robust transcriptional profiling. We then made several comparisons for differential gene expression (fold change 1.5, fdr=0.05) summarized in table 1. Our comparisons allowed us to group differentially expressed genes (DEGs) into 6 categories: (1) GR-regulated genes that are indifferent to AR-V7 status, (2) GR-regulated genes that are dependent on AR-V7 being present, (3) GR-regulated genes that are dependent on AR-V7 being absent, (4) AR-V7 regulated genes that are indifferent to GR activity, (5) AR-V7 regulated genes that are dependent on GR being active, and (6) AR-V7 regulated genes that are dependent on GR being inactive.

This analysis reveals that the presence or activity of one receptor has a profound impact on the ability of the other receptor to regulate gene expression. In 22RV1 cells, only 21% (141/664) of GR DEGs observed were indifferent to AR-V7 status. Similarly, only 17% (99/559) of AR-V7 DEGs observed were indifferent to GR activity. This effect is more pronounced in the VCaP cell line, where less than 2% (7/363) GR DEGs were observed to be indifferent to AR-V7 status, and

only 15% (66/422) AR-V7 DEGs were indifferent to GR activity. Notably, we observed 3-5 fold more GR DEGs when AR-V7 is absent compared to when AR-V7 is present. This pattern was also prevalent to a lesser extent with AR-V7 DEGs, where 20-65% more AR-V7 DEGs were found when GR signaling is inactive. While the precise mechanisms underlying these biological phenomena remain incompletely understood, these results demonstrate ARSI-resistant PC can exhibit distinct transcriptional profiles dependent on the status of both GR and AR-V7.

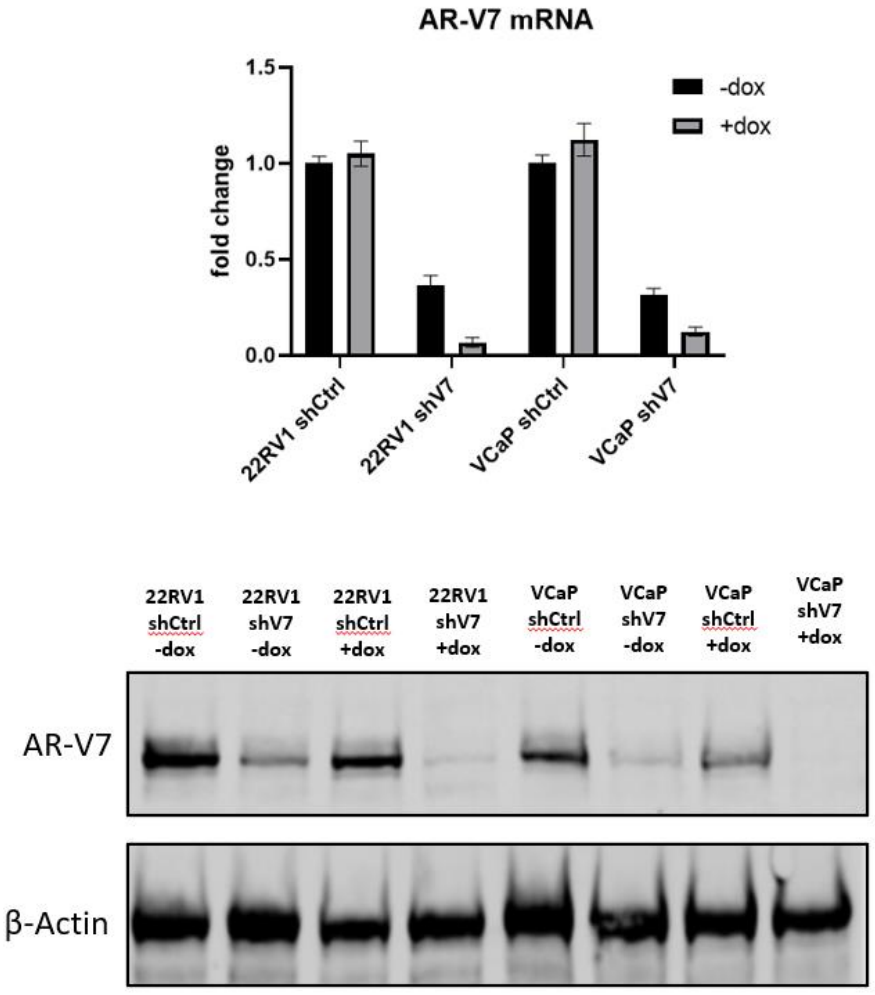

Figure 4.1 Knockdown of AR-V7 with shRNA in derivative cell lines.

Table 4.1 Comparisons for determining coordinated gene expression driven by GR and AR-V7

Control	Variable	Comparison
shV7RE	shV7RED	GR-regulated genes when AR-V7 is inactive
shCtrlRE	shCtrlRED	GR-regulated genes when AR-V7 is active
shV7RE	shCtrlRE	AR-V7-regulated genes when GR is inactive
shV7RED	shCtrlRED	AR-V7-regulated genes when GR is active

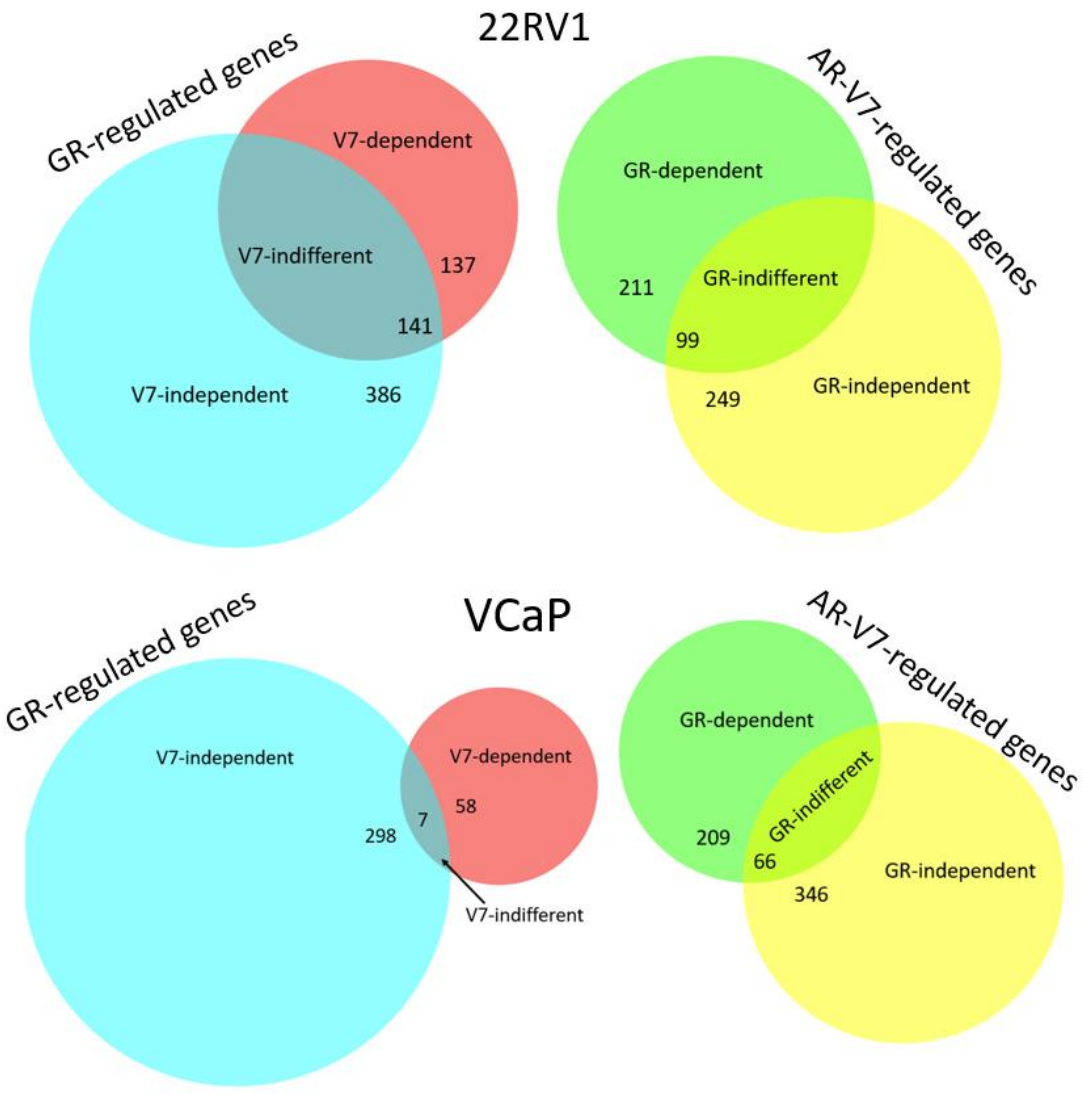

Figure 4.2 Differential gene expression driven by GR and AR-V7.

4.2.2 Coordinated activity between GR and AR-V7 promotes accelerated tumor progression following ARSI

RNA-seq analysis in 22RV1 shV7 and 22RV1 shCtrl cells revealed 325 genes that were differentially expressed only when both GR and AR-V7 were present and active. Naturally, we sought to determine whether this unique gene expression driven by GR and AR-V7 translated to any functional phenotypes. Ingenuity Pathway Analysis performed on this gene set returned enhanced growth and proliferation, as well as a suppression of apoptosis and cell death signaling as enriched pathways. Therefore, we selected proliferation as an appropriate phenotype to interrogate experimentally. Unsurprisingly, we found that AR-V7 alone and GR alone were sufficient to accelerate proliferation; however, the fastest rates of proliferation were observed when both GR and AR-V7 were active, while the slowest rates of proliferation occurred when both receptors were inactive. These experiments produced consistent results in both cell lines, and further support that together, GR and AR-V7 coordinate to drive a more potent proliferative phenotype than either receptor alone.

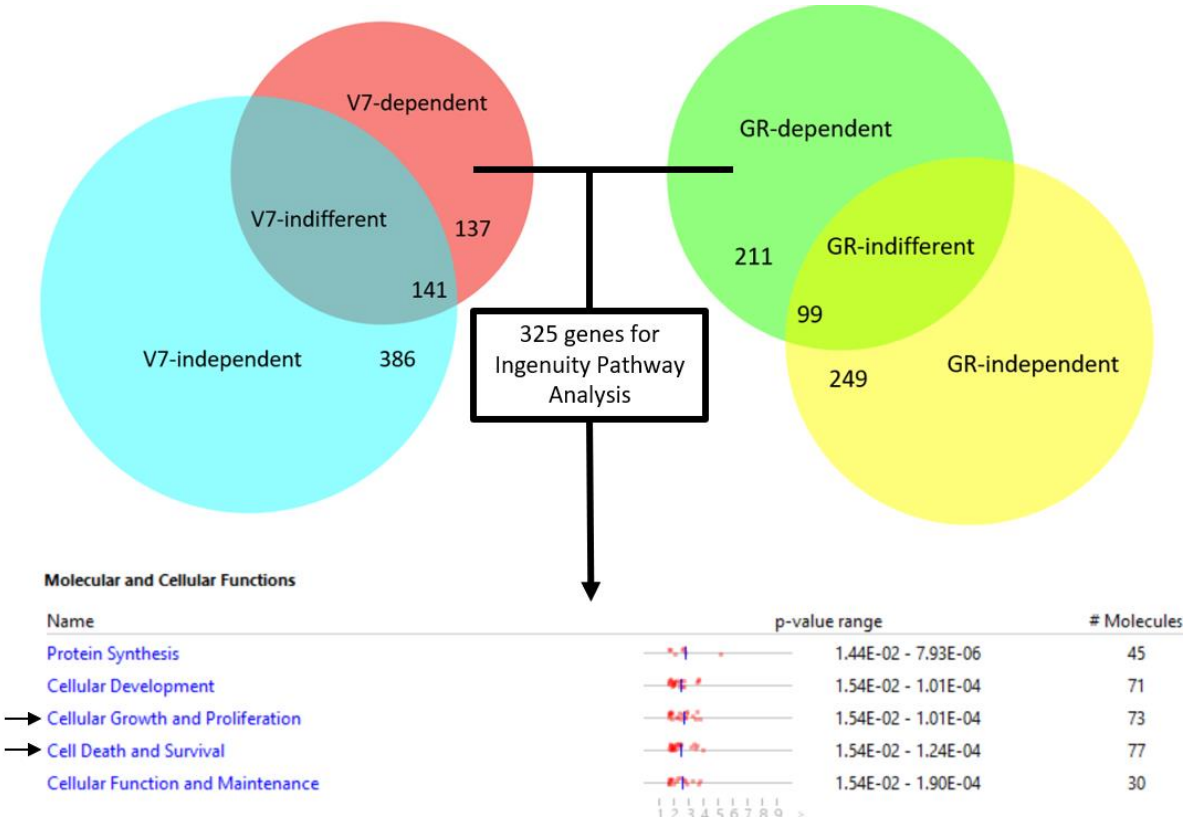

Figure 4.3 Ingenuity Pathway Analysis of 325 DEGs dependent on both GR and AR-V7 in 22RV1 cells identifies potential functional pathways impacted by coordinated activity between these receptors. Notably, this analysis returned enriched cellular growth and proliferation pathways as well as a suppression of cell death and survival pathways.

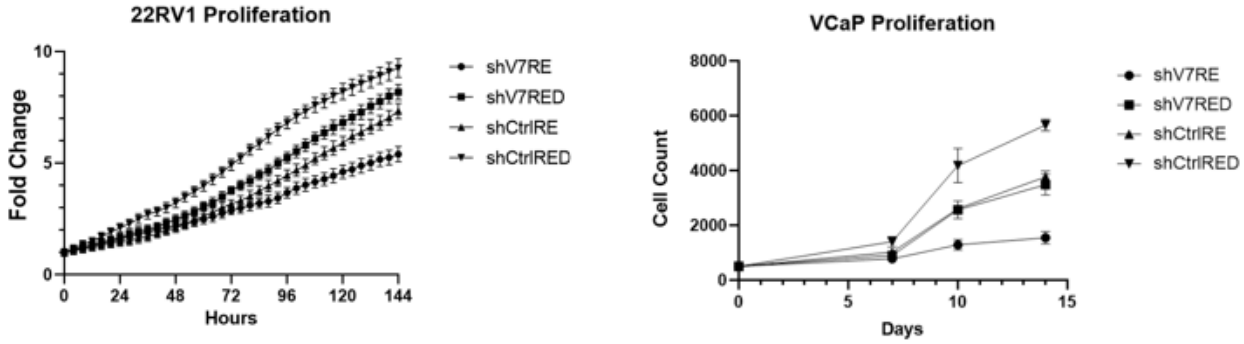

Figure 4.4 Coordinated activity between GR and AR-V7 drives accelerated proliferation in 22RV1 (left) and VCaP (right) derivative cells. While each receptor alone was able to confer resistance to ARSI, the receptors together produced a more potent phenotype.

4.3 Discussion

Following the detection of a novel endogenous interaction between GR and AR-V7, we report here that the presence and activity of one receptor dramatically impacts the ability of the other receptor to regulate gene expression. While several studies have investigated differential gene expression driven by GR or AR-V7 in PC, all have operated under the assumption that these receptors function independently. The extraordinary findings presented here directly challenge this long-held dogma in the field.

The goal of this project was to identify potential coordinated activity between GR and AR-V7. For this reason, we chose to focus our analysis primarily on conditions where both GR and AR-V7 were present and active. However, the status of these receptors varies considerably on a patient-to-patient basis [33,149]. Efforts should therefore be made to fully characterize the molecular mechanisms underlying the distinct transcriptional profiles driven by AR-V7 alone, GR alone, and coordinated activity between the two. Although the strongest proliferative phenotype was observed when both receptors were present and active, each receptor alone was still sufficient to maintain growth following ARSI. A complete understanding of these distinct signaling pathways and how each contributes to tumor progression could reveal unique vulnerabilities that can be exploited by novel therapeutic strategies.

The unique gene expression profile driven by coordinated activity between GR and AR-V7 is accompanied by a distinct phenotype: accelerated proliferation. This has obvious clinical implications and should be the subject of longitudinal studies to evaluate patient responses and outcomes on the basis of GR and AR-V7 status. If the potentiated growth we report here is observed clinically, the interaction between GR and AR-V7 could have utility as a biomarker to

predict tumor behavior. While no such test currently exists, single cell PLA of circulating tumor cells is an aspirational benchmark.

The decision to evaluate proliferation was informed by Ingenuity Pathway Analysis. While this approach revealed a relevant phenotype that we were able to validate experimentally, we almost certainly failed to capture the full range of biological effects mediated by coordinated activity between GR and AR-V7. Fortunately, the derivative cell lines generated from this work are valuable research tools and can be easily utilized for this purpose. Relatively simple assays evaluating clonogenic growth, anchorage-independent growth, migration, and invasion could all provide further insights into how these receptors drive resistance to ARSI.

Limitations in available systems to control AR-V7 expression and GR activity are a possible shortcoming in evaluating receptor co-dependence. Attempts to genomically delete *NR3C1* and *NR3C4* from our cell lines with CRISPR-Cas9 were not viable. Instead, this work utilized stably expressed shRNA to diminish AR-V7. However, more complete removal of AR-V7 may have enabled more robust characterization of potential phenotypes. Similarly, GR activity was diminished by withholding ligand or giving SGRM, which could lead to ligand-independent interactions between GR and AR-V7, although this was not observed in the experiments undertaken.

CHAPTER 5

SELECTIVE GLUCOCORTICOID RECEPTOR MODULATORS DISRUPT THE INTERACTION BETWEEN GR AND AR-V7

5.1 Introduction

Mechanisms of resistance to ARSI have emerged as promising therapeutic targets in PC, a concept best illustrated by the discovery of enz [105]. After determining that AR amplification confers resistance to first-generation antiandrogens, researchers performed a drug screen on nearly 200 thiohydantoin derivatives of the antiandrogen RU59063 to identify novel AR inhibitors [55]. Importantly, to overcome the problem of AR amplification, the drug screen was performed in cells engineered to express high levels of AR [55]. Enz demonstrated potent AR antagonism that was able to overcome AR amplification in pre-clinical models, and quickly progressed through clinical trials to achieve FDA approval [160].

The success of enz has inspired other approaches to target mechanisms of resistance. Amplification of the *MYCN* oncogene is observed in between 30-40% of NEPC and drives AR-independent tumor progression [104]. MYCN protein is stabilized through an allosteric interaction with Aurora kinase A that prevents protein degradation [161]. Targeting this interaction with the small molecule inhibitor alisertib disrupts the MYCN-Aurora A protein complex and significantly slows tumor progression in preclinical models of NEPC driven by MYCN [162].

Our lab identified a similar vulnerability when we discovered GR drives resistance to ARSI in PC [140]. GR regulates potent anti-inflammatory and immunosuppressive activity and for several decades has been a popular target for therapeutic intervention in a wide range of diseases [101]. Work from our lab has demonstrated that GR antagonism with SGRMs can effectively

antagonize GR signaling in PC and inhibit tumor progression in pre-clinical models [147]. Notably, many of these experiments demonstrating the efficacy of SGRMs were carried out in 22RV1 cells that express AR-V7. These efforts have resulted in two drugs, Relacorilant and CORT125128, progressing into Phase II clinical trials (NCT03674814, NCT03437941).

AR-V7 is unaffected by all FDA-approved therapies targeting AR, which either directly or indirectly target the AR LBD [94]. Several unique therapeutic strategies targeting AR-V7 have been proposed, including targeted degradation, small molecule inhibition of the D-box motif to prevent dimerization, the use of bromodomain inhibitors, as well as targeting downstream signaling that facilitates oncogenic activity [86]. However, each of these approaches has ultimately failed to reach approval for use in patients [86]. We hypothesize that a novel therapeutic strategy targeting AR-V7 indirectly with SGRMs could offer meaningful benefit to the subset of patients with AR-V7 and GR dual-expressing tumors who face poor clinical prognoses.

5.2 Results

Relying on several methodologies, we determined that SGRMs can effectively disrupt the interaction between GR and AR-V7. Using our NanoBRET system, we found GR antagonism with SGRMs significantly decreased the intensity of the interaction between GR and AR-V7, even in the presence of dexamethasone. In PC cell lines, we observed the addition of SGRMs significantly diminishes the endogenous interaction between GR and AR-V7 detectable by CoIP and PLA. Interestingly, the limited interaction between GR and AR-V7 following treatment with SGRMs was still localized primarily to the nucleus. Taken in conjunction with our previous preclinical work on GR antagonism, this discovery suggests SGRMs could have utility as an effective targeted therapeutic strategy for tumors expressing both GR and AR-V7.

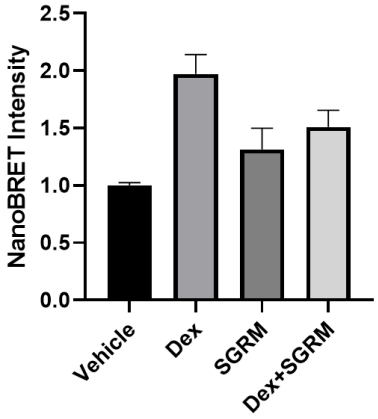

Figure 5.1 NanoBRET experiments demonstrate SGRMs significantly abrogate the interaction between GR and AR-V7.

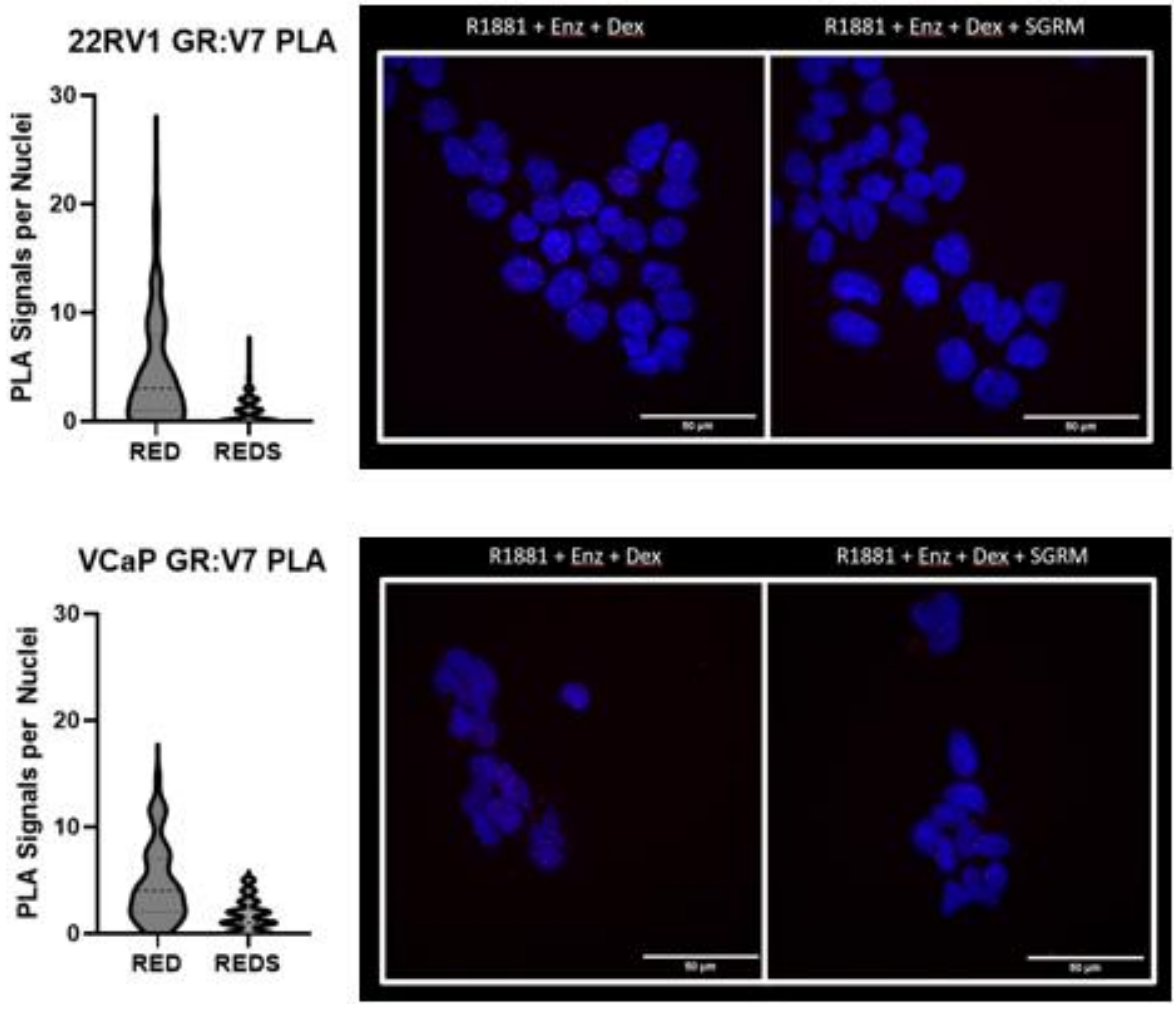

Figure 5. 2 Proximity Ligation Assays demonstrate SGRMs significantly abrogate the interaction between GR and AR-V7 in 22RV1 (top) and VCaP (bottom) cells.

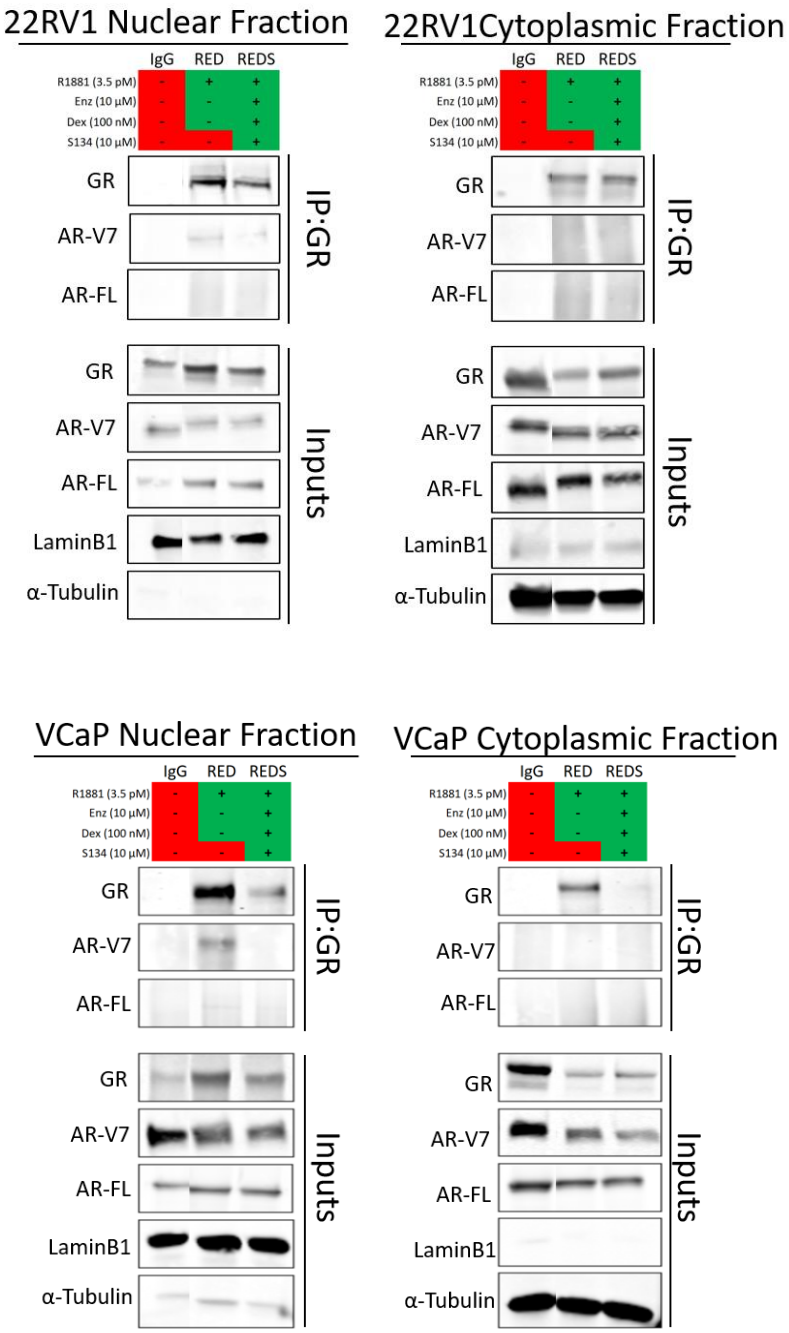

Figure 5.3 Co-immunoprecipitation assays following nuclear fractionation demonstrate SGRMs significantly abrogate the interaction between GR and AR-V7 in 22RV1 (top) and VCaP (bottom) cells.

5.3 Discussion

Patients with tumors expressing AR-V7 face poor clinical prognoses, as the lack of an LBD renders all current FDA-approved therapies ineffective [94,134]. Several attempts have been made to target AR-V7 signaling directly, but these efforts have ultimately failed for a variety of reasons [86]. Here, we provide rationale for a novel strategy to indirectly target AR-V7 signaling through antagonism of GR with SGRMs, an approach that effectively disrupts the interaction between the two receptors. Determining the extent to which SGRMs can reverse gene expression driven by coordinated activity between GR and AR-V7 is a crucial next step in evaluating their therapeutic potential in PC. Our lab is intimately involved in ongoing clinical trials for SGRMs in PC and retains privileged access to patient resources. Stratifying the patients from these trials based on AR-V7 status when interpreting results will provide early indications on the potential for SGRMs in this cohort. Circulating tumor cells collected before and after patients received SGRMs will be another invaluable resource uniquely available to our lab in the future. Robust profiling of these samples may reveal novel conserved mechanisms that allow PCs to overcome dual AR/GR antagonism.

Alternative approaches to antagonizing the novel interaction between GR and AR-V7 could also have meaningful therapeutic benefit. The class of small molecule inhibitors targeting D-box mediated dimerization are particularly promising in this regard [71]. The high conservation within the D-box motif has historically made drugging this target extremely difficult, with most compounds non-specifically inhibiting other NR3C-family receptors [151]. However, recent advances in computational approaches to drug design and improved crystallography have identified unique pockets within the AR DBD not found in other NR3C-family receptors [163]. Small molecule inhibitors designed to exploit this site have demonstrated the ability to selectively

impair AR dimerization [71]. Furthermore, these drugs have been shown to disrupt AR-V7 homodimerization and AR-V7 heterodimerization with AR-FL [151]. As no interaction between GR and AR-V7 has been previously reported, resolving whether D-box inhibitors can block this interaction is an important question for evaluating their clinical utility.

CHAPTER 6

THE MISSING LINK

6.1 Introduction

A critical component of this work was elucidating any relationship between GR and AR-V7 heterodimerization, and specific phenotypes that contribute towards therapeutic resistance and CRPC progression. We were able to robustly characterize the novel endogenous interaction between GR and AR-V7, and found its behavior is generally consistent with previous studies of NR3C-family receptors, notably, that AR-V7 and GR heterodimerize in a canonical D-box dependent manner and co-occupy DNA at the promoter of canonical AR target genes. Furthermore, we demonstrated that GR and AR-V7 drive distinct transcriptional programs dependent on each other, and that both receptors together drive a more potent proliferative phenotype. However, our investigation struggled to characterize the specific molecular mechanisms underlying this biology.

Next generation sequencing technology has revolutionized genomic research. Following the completion of the Human Genome Project in 2001, rapid advances led to the development of several new commercially available techniques for interrogating nucleic acids [164]. The cost of next-generation sequencing has fallen dramatically in the last 20 years, from nearly $100 million per genome to under $1000, allowing researchers unprecedented insight into cellular processes underlying disease [165]. Next-generation sequencing technologies have been adapted for several applications, probing the genome, transcriptome, cistrome, epigenome [164,165]. Successful implementation of these techniques is a critical step for defining the relationship between GR and AR-V7 in PC.

Our goal here was to define the AR-V7 and GR cistromes following ARSI using ChIP-seq. These cistromes could then be compared to corresponding transcriptomes revealing novel mechanisms through which AR-V7 and GR cooperate to drive resistance to ARSI. We were particularly interested in genomic regions containing peaks for both GR and AR-V7, as these represented candidate sites for GR and AR-V7 to co-occupy chromatin as a heterodimer. These sites could then be validated with ChIP-re-ChIP followed by qPCR.

We are not the first group to propose ChIP-Seq of either GR or AR-V7 in PC [125,129,130,131,132,133,140,141,142]. These receptors were initially established as independent drivers of CRPC progression, and thus the subject of considerable research to elucidate oncogenic signaling mechanisms [105]. However, because we were interested in GR and AR-V7 in the context of specific hormonal conditions, past studies were unsuitable for adaptation to our use. An early challenge we encountered for GR ChIP was the discontinuation of antibodies used previously in the literature [166]. Furthermore, at the time of performing experiments, the number of AR-V7 specific antibodies was limited to just two, Precision and RevMab, and neither antibody had been validated for use in ChIP-seq. Desperate to resolve the mechanisms governing GR and AR-V7 in PC, we pressed on undeterred; however, these experiments were ultimately unsuccessful.

6.2 Results

6.2.1 Sonication

To simplify the process of performing ChIP experiments, our lab purchased a Diagenode Bioruptor Pico for carrying out the process of sonication. Several experiments were performed on this machine to optimize chromatin shearing for downstream use. Variables considered for sonication included the total number of cycles, the duration of each cycle, the volume of lysate being sonicated, and the container holding lysates used for sonication. For both cell lines, optimal

chromatin shearing was achieved using 300µL lysates in a 1.5mL sonication tubes, and 10 cycles of 30 seconds on 30 seconds off. Consistent utilization of established sonication protocols is crucial for generating reproducible results.

6.2.2 Immunoprecipitation

After optimizing sonication, we next interrogated whether the antibodies in our toolbox were suitable for immunoprecipitating our proteins of interest. The strong constitutive expression of GR and AR-V7 led us first optimize our ChIP protocol in 22RV1 cell lines. Two antibodies targeting AR-V7 and three antibodies targeting GR were selected for evaluation. We established that both the Precision and RevMab antibodies were able to immunoprecipitate AR-V7 as confirmed by western blotting. Similarly, each GR antibody demonstrated the ability to immunoprecipitate GR to an extent. We chose to proceed with the Precision AR-V7 antibody and the D8H2 GR antibody, as these demonstrated sufficient cost-effective immunoprecipitation.

6.2.3 ChIP-PCR

qPCR of eluted ChIP DNA is an important quality control step for these experiments. We therefore selected the promoters of the canonical AR target genes KLK3 and FKBP5 to probe for AR-V7 and GR occupancy. Previous studies have independently established these regions as binding sites for AR-V7, GR, and AR-FL. Following immunoprecipitation with the Precision antibody, AR-V7 occupancy was detected by qPCR at both sites in 22RV1 cells. This phenomenon was also observed following immunoprecipitation of GR with the D8H2 antibody. Notably, both GR and AR-V7 demonstrated higher occupancy at these sites when GR was liganded. These data indicate successful elution of ChIP DNA and prompted the sending of eluted DNA samples to the University of Chicago Functional Genomics core for library preparation and sequencing.

Figure 6.1 Antibody validation for GR and AR-V7 ChIP. Two AR-V7 antibodies (RevMab, Precision) and three GR antibodies (D8H2, BUGR2, G-5) were used for immunoprecipitation. For each experiment, 3µg of primary antibody was used.

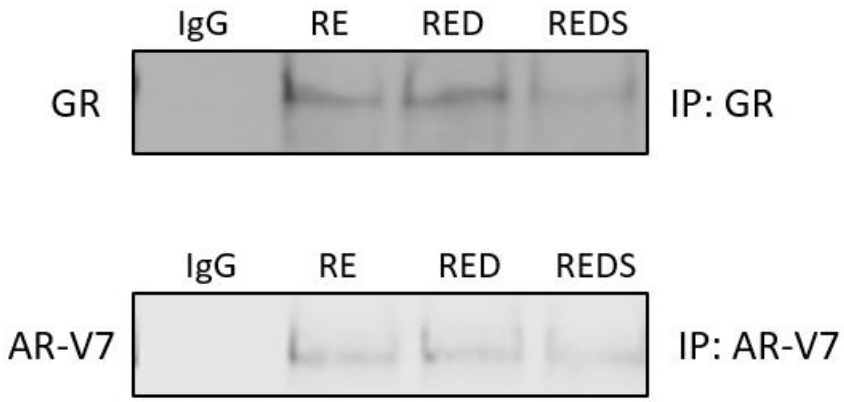

Figure 6.2 Immunoprecipitation of GR and AR-V7 in 22RV1 cells for ChIP experiments.

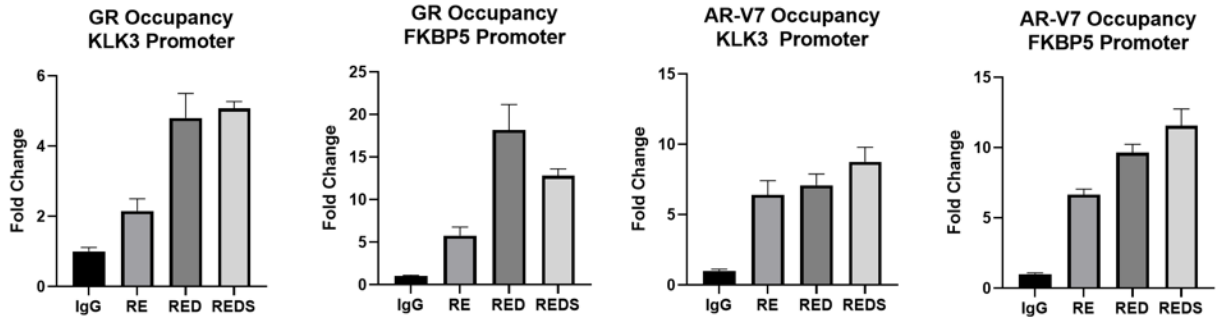

Figure 6.3 ChIP-PCR revealed AR-V7 and GR occupancy at the promoters of canonical AR target genes. As expected, GR occupancy increased following the addition of its ligand dexamethasone.

6.2.4 ChIP-Seq

The fundamental goal of these experiments was to define the GR and AR-V7 cistrome through next-generation sequencing of eluted ChIP DNA. Despite passing every quality control measure along the way, our attempts to sequence eluted ChIP DNA ultimately failed. The first indication of unsuccessful GR ChIP-seq was the number of peaks observed. When GR was liganded following ARSI, bioinformatic analysis indicated over 10,000 peaks were observed, more than twice the number previously detected in PC [141,142]. Furthermore, roughly four times as many GR binding peaks were observed when GR was unliganded, an observation inconsistent with known GR behavior [101,136]. Despite the high number of GR peaks, none were observed at several canonical GR binding sites, including the SGK1 promoter, FKBP5 promoter, and KLK3 promoter. AR-V7 ChIP also suffered from having an inordinate number of peaks. Where previous studies have reported several hundred to a few thousand AR-V7 binding sites, our bioinformatic analysis called over 100,000 AR-V7 peaks in each condition. Similar to GR, AR-V7 occupancy was not observed at canonical binding sites, including the FKBP5 and UBE2C promoter. For these reasons, we concluded that our ChIP-seq experiments lack sufficient quality to draw meaningful conclusions.

6.3 Discussion

Failure to successfully characterize the AR-V7 and GR cistromes was the major shortcoming of this project. However, the work done here does provide a meaningful foundation for pursuing this goal in the future. We have established valuable standardized protocols for sonication, immunoprecipitation, and elution that have each passed traditional quality control tests. These small successes will significantly simplify experimental procedures for future researchers seeking to answer these important questions. Despite this series of failures, full characterization of

the GR and AR-V7 cistromes in PC remains an important goal and should continue to be pursued. This could potentially be facilitated through collaboration with other groups that have managed to successfully perform AR-V7 ChIP-seq or GR ChIP-seq. Alternatively, more sensitive techniques for evaluating protein-DNA interactions could be implemented, such as ChIP-exo-seq or CUT&RUN-seq [167,168]. Importantly, these techniques will still require capable primary antibodies for targeting the proteins of interest. One step in our procedure where we currently lack sufficient quality control metrics is library preparation. Eluted ChIP DNA is sent to the University of Chicago Functional Genomics core for library preparation and subsequent sequencing. However, quality control metrics for library preparation are not returned. Generating our own libraries and assessing them for quality before sequencing could identify the specific step where these experiments have failed.

CHAPTER 7

SIGNIFICANCE AND FUTURE DIRECTIONS

7.1 Significance

PC is the second leading cause of cancer-related death in American men [1]. Diagnosis at a local or regional stage is associated with excellent prognoses, and advances in screening and early detection have improved overall survival [1]. However, the outlook for men that have advanced disease remains bleak, with five-year survival rates dropping to only 28% [25]. For decades, androgen deprivation has been the standard of care for patients with biochemical recurrence after primary therapy, locally advanced disease, or metastatic disease [41]. While this approach provides initial benefit, the majority of these men progress to CRPC within 2-3 years [41]. At this stage, the disease is considered incurable, and treatment is designed to reduce tumor burden, alleviate symptoms, and prolong overall survival [105]. A hallmark of CRPC is a restoration of androgen signaling, and next-generation antiandrogens can offer temporary benefit to many of these patients [105]. However, CRPC inevitably develops various mechanisms of resistance allowing for tumor progression, highlighting a major clinical challenge faced by oncologists. Understanding these mechanisms of resistance, and any therapeutic vulnerabilities they may present, is crucial for improving outcomes for patients with CRPC.

Our lab has made several notable contributions towards resolving the role of GR in mediating resistance to ARSI in CRPC. Past studies revealed GR has its expression upregulated in response to ARSI and can bypass AR to partially restore canonical androgen signaling pathways that maintain tumor progression [140]. Preclinical investigations into GR antagonism in the context of CRPC found SGRMs were able to effectively antagonize GR signaling in the prostate

and slow tumor progression following ARSI [147]. These efforts culminated in two SGRMs progressing to clinical trials where their effects are currently being evaluated (NCT03674814, NCT03437941). During the course of a standard ChIP-Seq experiment intended to characterize the GR cistrome in PC, an N-terminal AR antibody detected AR-V7 but not AR-FL on quality control western blots of immunoprecipitated GR. Work from several other groups had previously established a role for AR-V7 promoting resistance to ARSI [94]. However, this mechanism was proposed to function independently of GR [149]. Co-immunoprecipitation of GR and AR-V7 was a direct challenge to this dogma and the foundation for this dissertation.

Robust characterization of AR-V7 dimerization revealed several novel insights. Dimerization is an established prerequisite for NR3C-family receptor activity and is mediated by the conserved D-box motif within the DBD [90]. Homodimerization is favored by liganded NR3C-family receptors, where distinct CTD architecture initiates specific contacts between dimers allowing for discrimination between various family members [90]. The unique biology of AR-V7 allows it to overcome these limitations and promiscuously heterodimerize with liganded NR3C-family receptors in a canonical D-Box dependent manner. This discovery challenges the findings of numerous previous investigations into AR-V7 dimerization, which have been limited to AR-V7 homodimerization and heterodimerization with AR-FL [129-133]. These studies mostly relied on the absence of AR-FL to characterize AR-V7 homodimerization, a metric this work suggests is insufficient.

Physiological conditions of CRPC restrict AR-FL nuclear translocation, creating an unfavorable environment for heterodimerization with AR-V7 [153]. Conversely, this environment is ideal for GR to emerge as an alternative partner for heterodimerization with AR-V7. In two PC cell lines with varied and clinically relevant AR/GR characteristics, we consistently detected an

endogenous nuclear interaction between GR and AR-V7 following ARSI. Importantly, we recorded significantly more interactions with AR-V7 when GR was liganded by dexamethasone. These observations support a model where AR-V7 dimerization is determined by the viable partners available to it within a given cell. The development of new methods to discriminate between AR-V7 homodimerization and heterodimerization with various NR3C-receptors is imperative for full characterization of the AR-V7 landscape in PC.

An interaction between GR and AR-V7 is notable for the role each receptor plays in CRPC progression. Remarkably, we found that together these receptors coordinate to drive a distinct gene expression profile. This discovery contradicts the established view within the PC field that AR-V7 and GR drive resistance to ARSI independently [149]. While both GR and AR-V7 were sufficient to drive resistance to ARSI alone, together they drive a much stronger proliferative phenotype. RNA-seq revealed unique transcriptional profiles for resistance driven by GR alone, AR-V7 alone, and both receptors together. While we chose to prioritize our analysis in the condition where both receptors were present and active, understanding the unique biology driving resistance to ARSI in each context could reveal novel therapeutic vulnerabilities to exploit.

Although we were able to characterize the interaction between GR and AR-V7 in CRPC, and clearly demonstrated that coordinated activity between these receptors drives a potent oncogenic phenotype, this work struggled to uncover the molecular mechanisms linking these together. A major shortcoming in this regard was the failure to define the complete GR and AR-V7 cistromes. Capturing the shared cistrome between these receptors would reveal candidate sites for genomic co-occupancy by AR-V7 and GR. These could then be probed with ChIP-re-ChIP-PCR to expand the number of validated sites shared by GR and AR-V7. It is also notable that dimerization is not necessarily the sole mechanism driving coordinated activity between GR and

AR-V7. Through interactions with the SWI/SNF complex GR is an established pioneer factor that can remodel the global chromatin landscape [102]. This could theoretically open up novel sites for AR-V7 to bind and regulate gene expression. ATAC-Seq can be utilized to generate a snapshot of the epigenome in this context. Data from ChIP-Seq and ATAC-Seq experiments could then be coupled with RNA-Seq to define distinct molecular mechanisms responsible for the accelerated proliferation reported here.

Exploiting mechanisms of resistance to ARSI for therapeutic benefit is a promising strategy for treating CRPC [105]. AR-V7 represents an obvious target, as it is easily detectable in patients and directly linked to poor outcomes [134]. However, attempts to antagonize AR-V7 are complicated by the loss of its LBD, and these approaches have failed to gain traction in the clinic [86]. Recently, GR antagonism with SGRMs has emerged as a viable strategy for treating a subset of patients whose tumors express GR, and trials with these drugs are ongoing [147]. This work found that SGRMs are able to effectively disrupt the interaction between GR and AR-V7. Clinically, AR-V7 is used as a biomarker to predict responses to therapy, and frequently indicates general chemotherapy with docetaxel as the optimal approach [169]. Delaying progression with targeted approaches is preferable, and indirect inhibition of AR-V7 activity through GR antagonism with SGRMs could provide meaningful benefit to the subset of patients with dual positive tumors. Further work is needed to establish the full capabilities of SGRMs to reverse oncogenic signaling driven by coordinated activity between GR and AR-V7. Overall, the scientific contributions described in this work provide crucial insights into the complexities of hormone receptor biology in CRPC.

7.2 Future Directions

7.2.1 The Complete AR-V7 Dimerization Landscape in Prostate Cancer

AR-V7 dimerization has been a subject of rigorous debate within the PC fie for over a decade [94]. Conflicting evidence has shifted expert opinion over time between various models either favoring homodimerization or heterodimerization with AR-FL. However, the ability of AR-V7 to promiscuously heterodimerize with a full suite of NR3C-family receptors has shifted the paradigm rendering previous interpretations obsolete. Therefore, a complete characterization of the AR-V7 dimerization landscape in PC is vital for understanding its full oncogenic signaling potential in this context.

Central to resolving this problem is understanding what governs AR-V7 dimerization. To this effect, our NanoBRET system can be implemented to measure dimerization kinetics. Interactions between a halo-tagged NR3C-family receptor and AR-V7 establish baseline levels of interaction, while different co-transfected receptors lacking a halo-tag can compete for dimerization with AR-V7. Changes to NanoBRET signal intensity relative to baseline are then used to extrapolate a dimerization affinity between AR-V7 and each NR3C-family receptor.

7.2.2 Therapeutic Vulnerabilities Dependent on GR and AR-V7 Status

Next generation sequencing can provide unprecedented insight into the molecular mechanisms governing human disease. Our RNA-seq experiments revealed unique transcriptional profiles dependent on the status of both AR-V7 and GR. Here, we focused our analysis on the co-dependent state in which both receptors were expressed and active. However, there is significant benefit to gain from expanding the scope to include a comprehensive characterization of the full molecular landscape in PC. Matching the cistromes, transcriptomes, and epigenomes in the context of AR-V7 and GR could reveal unique molecular vulnerabilities for novel therapeutic strategies.

The adaptive nature of CRPC can make treatment selection difficult. Needle biopsies are informative, but invasive to patients and not practical for regular monitoring [170]. PSA can be used to monitor tumor progression and responses to treatment, but it offers little information about the molecular drivers of disease [171]. The development of non-invasive liquid biopsies has filled an important role, allowing for molecular features of tumors to predict responses to treatment [171]. This has been successfully implemented in the clinical setting of PC, with AR-V7 mRNA from CTCs predicting responses to enz and abi [134]. A landmark goal of the field would be to define markers or gene signatures from CTCs that can determine the primary oncogenic driver of a patient's tumor and indicate specific therapeutic strategies to optimize patient responses.

7.2.3 The Relationship Between GR and HoxB13

A role for HoxB13 first emerged in PC when a recurrent G84E mutation was found in a subset of familial PCs [172]. Subsequent research into the mechanisms of HoxB13 found that it acts as a bivalent regulator of AR chromatin binding and confers context-dependent activity [173]. In CRPC, HoxB13 expression tends to promote growth through E2F activation and cell cycle progression [174]. Recent studies have revealed that the AR-V7 cistrome is governed by HoxB13 [130,133]. However, no relationship between HoxB13 and GR has been established. In the context of AR-V7, HoxB13 could either facilitate heterodimerization with GR or exclude GR to favor other dimerization partners. Understanding these relationships will be critical in uncovering the complete molecular mechanisms underlying AR-V7 signaling in CRPC.

7.2.4 A Role for AR-V7 in Breast Cancer

Despite arising in organs that are vastly different in terms of anatomy and physiological function, PC and breast cancer are remarkably similar [175]. These tumors are typically hormone-dependent, responsive to endocrine therapies, and activate similar oncogenic signaling pathways to promote tumor progression [175]. Just as the administration of estrogens suppresses PC growth, so too does giving androgens to patients with breast cancer [175]. Interestingly, GR plays a similar context-dependent role in both tumor types, while AR behaves similar to GR in breast cancer [176]. High AR/GR expression is associated with better clinical outcomes in ER-positive breast cancer patients, but worse outcomes in ER-negative breast cancer [177,178]. In this sense, ER-negative breast cancer and CRPC behave strikingly similar, where signaling from the primary oncogenic driver is ab sent or blocked, and compensatory GR/AR signaling drives tumor progression. This observation suggests these tumors could be vulnerable to similar therapeutic strategies, and indeed SGRMs have been proposed for the treatment of GR-expressing triple negative breast cancer [178].

The decisive role of AR-V7 in CRPC progression has naturally made it a protein of interest in breast cancer research. Early studies detected AR-V7 mRNA in over 50% of primary breast cancers, although it was only found at the protein level in 3/54 tumors [179]. Notably, each tumor expressing AR-V7 at the protein level was ER-negative [179]. A more recent investigation found AR-V7 mRNA and protein was detectable in nearly 10% of all breast cancer [180]. Unlike PC, where an plethora of readily available cell lines can express clinically relevant features of AR-V7, breast cancer research is handicapped by the lack of relevant model systems [175,180]. However, that does not mean this endeavor is not worth pursuing; the clear link between AR-V7 and CRPC progression should make understanding its role in breast cancer a priority for future study.

REFERENCES

1. Lee, Christine, Oluyemi Akin-Olugbade, and Alexander Kirschenbaum. "Overview of Prostate Anatomy, Histology, and Pathology." *Endocrinology and Metabolism Clinics of North America*, Hormones and Cancer: Breast and Prostate, 40, no. 3 (September 1, 2011): 565–75. https://doi.org/10.1016/j.ecl.2011.05.012.

2. Nutton, Vivian. *Ancient Medicine*. 2nd ed. London: Routledge, 2012. https://doi.org/10.4324/9780203081297.

3. Palmer, Richard. "Nicolò Massa, His Family and His Fortune." *Medical History* 25, no. 4 (October 1981): 385–410. https://doi.org/10.1017/S0025727300034888.

4. Saunders, J. B., and Charles O'Malley. *The Illustrations from the Works of Andreas Vesalius of Brussels*. Courier Corporation, 2013.

5. Josef Marx, Franz, and Axel Karenberg. "History of the Term Prostate." *The Prostate* 69, no. 2 (2009): 208–13. https://doi.org/10.1002/pros.20871.

6. McNeal, John E. "The Zonal Anatomy of the Prostate." *The Prostate* 2, no. 1 (1981): 35–49. https://doi.org/10.1002/pros.2990020105.

7. Aumüller, G., and J. Seitz. "Protein Secretion and Secretory Processes in Male Accessory Sex Glands." In *International Review of Cytology*, edited by K. W. Jeon and M. Friedlander, 121:127–231. Academic Press, 1990. https://doi.org/10.1016/S0074-7696(08)60660-9.

8. Hayward, Simon W., and Gerald R. Cunha. "The Prostate: Development and Physiology." *Radiologic Clinics of North America* 38, no. 1 (January 1, 2000): 1–14. https://doi.org/10.1016/S0033-8389(05)70146-9.

9. Levin, R. J. "Prostate-Induced Orgasms: A Concise Review Illustrated with a Highly Relevant Case Study." *Clinical Anatomy* 31, no. 1 (2018): 81–85. https://doi.org/10.1002/ca.23006.

10. Hiort, Olaf. "The Differential Role of Androgens in Early Human Sex Development." *BMC Medicine* 11 (June 24, 2013): 152. https://doi.org/10.1186/1741-7015-11-152.

11. Denmeade, Samuel R., and John T. Isaacs. "A History of Prostate Cancer Treatment." *Nature Reviews Cancer* 2, no. 5 (May 2002): 389–96. https://doi.org/10.1038/nrc801.

12. Whitney, Charles. "Malignant Disease of the Prostate, with Report of a Case of Carcinoma." https://www.nejm.org/doi/full/10.1056/NEJM189304131281502.

13. Lepor, Herbert. "A Review of Surgical Techniques for Radical Prostatectomy." *Reviews in Urology* 7, no. Suppl 2 (2005): S11–17.

14. Lederman, Manuel. “The Early History of Radiotherapy: 1895–1939.” *International Journal of Radiation Oncology*Biology*Physics* 7, no. 5 (May 1, 1981): 639–48. https://doi.org/10.1016/0360-3016(81)90379-5.

15. Young, Hugh Hampton. “Radium Therapy in Cancer of the Prostate and Bladder.” https://jamanetwork.com/journals/jama/article-abstract/442552.

16. Ward, Matthew C., Rahul D. Tendulkar, Jay P. Ciezki, and Eric A. Klein. “Future Directions From Past Experience: A Century of Prostate Radiotherapy.” *Clinical Genitourinary Cancer* 12, no. 1 (February 1, 2014): 13–20. https://doi.org/10.1016/j.clgc.2013.08.003.

17. Hunter, John. *The Works of John Hunter, F.R.S.: With Notes*. Edited by James F. Palmer. Vol. 2. Cambridge Library Collection - History of Medicine. Cambridge: Cambridge University Press, 2015. https://doi.org/10.1017/CBO9781316034804.

18. Moore, Robert A., and Allister M. McLellan. “A Histological Study of the Effect of the Sex Hormones on the Human Prostate1.” *Journal of Urology* 40, no. 5 (November 1938): 641–57. https://doi.org/10.1016/S0022-5347(17)71807-1.

19. Gutman, Alexander, and Ethel Gutman. “AN ‘ ACID ’ PHOSPHATASE OCCURRING IN THE SERUM OF PATIENTS WITH METASTASIZING CARCINOMA OF THE PROSTATE GLAND.” https://www.jci.org/articles/view/100974.

20. Huggins, Charles, and Philip Johnson Clark. “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.” *Journal of Experimental Medicine* 72, no. 6 (December 1, 1940): 747–62. https://doi.org/10.1084/jem.72.6.747.

21. Huggins, Charles, and Philip Johnson Clark. “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.” *Journal of Experimental Medicine* 72, no. 6 (December 1, 1940): 747–62. https://doi.org/10.1084/jem.72.6.747.

22. Pernar, Claire H., Ericka M. Ebot, Kathryn M. Wilson, and Lorelei A. Mucci. “The Epidemiology of Prostate Cancer.” *Cold Spring Harbor Perspectives in Medicine* 8, no. 12 (December 1, 2018): a030361. https://doi.org/10.1101/cshperspect.a030361.

23. Sung, Hyuna, Jacques Ferlay, Rebecca L. Siegel, Mathieu Laversanne, Isabelle Soerjomataram, Ahmedin Jemal, and Freddie Bray. “Global Cancer Statistics 2020: GLOBOCAN Estimates of Incidence and Mortality Worldwide for 36 Cancers in 185 Countries.” *CA: A Cancer Journal for Clinicians* 71, no. 3 (2021): 209–49. https://doi.org/10.3322/caac.21660.

24. Cai, Qiliang, Yegang Chen, Dingrong Zhang, Jiancheng Pan, Zunke Xie, Chenjie Xu, Shu Li, et al. “Estimates of Over-Time Trends in Incidence and Mortality of Prostate Cancer from 1990 to 2030.” *Translational Andrology and Urology* 9, no. 2 (April 2020): 196–209. https://doi.org/10.21037/tau.2020.02.21.

25. “Key Statistics for Prostate Cancer | Prostate Cancer Facts.” https://www.cancer.org/cancer/prostate-cancer/about/key-statistics.html.

26. Zhou, Cindy Ke, David P. Check, Joannie Lortet-Tieulent, Mathieu Laversanne, Ahmedin Jemal, Jacques Ferlay, Freddie Bray, Michael B. Cook, and Susan S. Devesa. “Prostate Cancer Incidence in 43 Populations Worldwide: An Analysis of Time Trends Overall and by Age Group.” *International Journal of Cancer* 138, no. 6 (March 15, 2016): 1388–1400. https://doi.org/10.1002/ijc.29894.

27. Center, Melissa M., Ahmedin Jemal, Joannie Lortet-Tieulent, Elizabeth Ward, Jacques Ferlay, Otis Brawley, and Freddie Bray. “International Variation in Prostate Cancer Incidence and Mortality Rates.” *European Urology* 61, no. 6 (June 1, 2012): 1079–92. https://doi.org/10.1016/j.eururo.2012.02.054.

28. Powell, Isaac J. “Epidemiology and Pathophysiology of Prostate Cancer in African-American Men.” *Journal of Urology* 177, no. 2 (February 2007): 444–49. https://doi.org/10.1016/j.juro.2006.09.024.

29. Sonn, G. A., W. Aronson, and M. S. Litwin. “Impact of Diet on Prostate Cancer: A Review.” *Prostate Cancer and Prostatic Diseases* 8, no. 4 (December 2005): 304–10. https://doi.org/10.1038/sj.pcan.4500825.

30. Wolk, Alicja. “Diet, Lifestyle and Risk of Prostate Cancer.” *Acta Oncologica* 44, no. 3 (May 1, 2005): 277–81. https://doi.org/10.1080/02841860510029572.

31. Papadopoulos, Georgios, Dimitrios Delakas, Lydia Nakopoulou, and Theodoros Kassimatis. “Statins and Prostate Cancer: Molecular and Clinical Aspects.” *European Journal of Cancer* 47, no. 6 (April 1, 2011): 819–30. https://doi.org/10.1016/j.ejca.2011.01.005.

32. Zhen, Jun Tu, Jamil Syed, Kevin Anh Nguyen, Michael S. Leapman, Neeraj Agarwal, Karina Brierley, Xavier Llor, Erin Hofstatter, and Brian Shuch. “Genetic Testing for Hereditary Prostate Cancer: Current Status and Limitations.” *Cancer* 124, no. 15 (2018): 3105–17. https://doi.org/10.1002/cncr.31316.

33. Robinson, Dan, Eliezer M. Van Allen, Yi-Mi Wu, Nikolaus Schultz, Robert J. Lonigro, Juan-Miguel Mosquera, Bruce Montgomery, et al. “Integrative Clinical Genomics of Advanced Prostate Cancer.” *Cell* 161, no. 5 (May 21, 2015): 1215–28. https://doi.org/10.1016/j.cell.2015.05.001.

34. St John, Jason, Katelyn Powell, M. Katie Conley-Lacomb, and Sreenivasa R. Chinni. “TMPRSS2-ERG Fusion Gene Expression in Prostate Tumor Cells and Its Clinical and Biological Significance in Prostate Cancer Progression.” *Journal of*

Cancer Science & Therapy 4, no. 4 (April 26, 2012): 94–101. https://doi.org/10.4172/1948-5956.1000119.

35. Klezovitch, Olga, Michael Risk, Ilsa Coleman, Jared M. Lucas, Manda Null, Lawrence D. True, Peter S. Nelson, and Valeri Vasioukhin. "A Causal Role for ERG in Neoplastic Transformation of Prostate Epithelium." *Proceedings of the National Academy of Sciences of the United States of America* 105, no. 6 (February 12, 2008): 2105–10. https://doi.org/10.1073/pnas.0711711105.

36. Chodak, Gerald W., Paul Keller, and Harry W. Schoenberg. "Assessment of Screening for Prostate Cancer Using the Digital Rectal Examination." *The Journal of Urology* 141, no. 5 (May 1, 1989): 1136–38. https://doi.org/10.1016/S0022-5347(17)41192-X.

37. Stamey, Thomas A., Norman Yang, Alan R. Hay, John E. McNeal, Fuad S. Freiha, and Elise Redwine. "Prostate-Specific Antigen as a Serum Marker for Adenocarcinoma of the Prostate." *New England Journal of Medicine* 317, no. 15 (October 8, 1987): 909–16. https://doi.org/10.1056/NEJM198710083171501.

38. Catalona, W. J., D. S. Smith, T. L. Ratliff, K. M. Dodds, D. E. Coplen, J. J. Yuan, J. A. Petros, and G. L. Andriole. "Measurement of Prostate-Specific Antigen in Serum as a Screening Test for Prostate Cancer." *The New England Journal of Medicine* 324, no. 17 (April 25, 1991): 1156–61. https://doi.org/10.1056/NEJM199104253241702.

39. Etzioni, Ruth, David F. Penson, Julie M. Legler, Dante di Tommaso, Rob Boer, Peter H. Gann, and Eric J. Feuer. "Overdiagnosis Due to Prostate-Specific Antigen Screening: Lessons From U.S. Prostate Cancer Incidence Trends." *JNCI: Journal of the National Cancer Institute* 94, no. 13 (July 3, 2002): 981–90. https://doi.org/10.1093/jnci/94.13.981.

40. Constantinou, J., and M. R. Feneley. "PSA Testing: An Evolving Relationship with Prostate Cancer Screening." *Prostate Cancer and Prostatic Diseases* 9, no. 1 (March 2006): 6–13. https://doi.org/10.1038/sj.pcan.4500838.

41. Litwin, Mark S., and Hung-Jui Tan. "The Diagnosis and Treatment of Prostate Cancer: A Review." *JAMA* 317, no. 24 (June 27, 2017): 2532–42. https://doi.org/10.1001/jama.2017.7248.

42. Matlaga, BRIAN R., L. ANDREW Eskew, and DAVID L. McCULLOUGH. "Prostate Biopsy: Indications and Technique." *The Journal of Urology* 169, no. 1 (January 1, 2003): 12–19. https://doi.org/10.1016/S0022-5347(05)64024-4.

43. Egevad, L., T. Granfors, L. Karlberg, A. Bergh, and P. Stattin. "Prognostic Value of the Gleason Score in Prostate Cancer." *BJU International* 89, no. 6 (2002): 538–42. https://doi.org/10.1046/j.1464-410X.2002.02669.x.

44. Bangma, Chris H., and Monique J. Roobol. “Defining and Predicting Indolent and Low Risk Prostate Cancer.” *Critical Reviews in Oncology/Hematology* 83, no. 2 (August 1, 2012): 235–41. https://doi.org/10.1016/j.critrevonc.2011.10.003.

45. Zlotta, Alex, and Frans M. J. Debruyne. “Expert Opinion on Optimal Testosterone Control in Prostate Cancer.” *European Urology Supplements*, Expert Report on Optimising Hormonal Control in Prostate Cancer, 4, no. 8 (November 1, 2005): 37–41. https://doi.org/10.1016/j.eursup.2005.08.005.

46. Zirkin, Barry R, and Vassilios Papadopoulos. “Leydig Cells: Formation, Function, and Regulation†.” *Biology of Reproduction* 99, no. 1 (July 1, 2018): 101–11. https://doi.org/10.1093/biolre/ioy059.

47. Schally, Andrew V., Norman L. Block, and Ferenc G. Rick. “Discovery of LHRH and Development of LHRH Analogs for Prostate Cancer Treatment.” *The Prostate* 77, no. 9 (2017): 1036–54. https://doi.org/10.1002/pros.23360.

48. Gomella, Leonard G. “Effective Testosterone Suppression for Prostate Cancer: Is There a Best Castration Therapy?” *Reviews in Urology* 11, no. 2 (2009): 52–60.

49. Kirby, M., C. Hirst, and E. D. Crawford. “Characterising the Castration-Resistant Prostate Cancer Population: A Systematic Review.” *International Journal of Clinical Practice* 65, no. 11 (2011): 1180–92. https://doi.org/10.1111/j.1742-1241.2011.02799.x.

50. Goldspiel, Barry R., and David R. Kohler. “Flutamide: An Antiandrogen for Advanced Prostate Cancer.” *DICP* 24, no. 6 (June 1, 1990): 616–23. https://doi.org/10.1177/106002809002400612.

51. Goa, Karen L., and Caroline M. Spencer. “Bicalutamide in Advanced Prostate Cancer.” *Drugs & Aging* 12, no. 5 (May 1, 1998): 401–22. https://doi.org/10.2165/00002512-199812050-00006.

52. Dole, Ernest J, and Mark T Holdsworth. “Nilutamide: An Antiandrogen for the Treatment of Prostate Cancer.” *Annals of Pharmacotherapy* 31, no. 1 (January 1, 1997): 65–75. https://doi.org/10.1177/106002809703100112.

53. Lam, John S., John T. Leppert, Sreenivas N. Vemulapalli, Oleg Shvarts, and Arie S. Belldegrun. “Secondary Hormonal Therapy for Advanced Prostate Cancer.” *The Journal of Urology* 175, no. 1 (January 1, 2006): 27–34. https://doi.org/10.1016/S0022-5347(05)00034-0.

54. Culig, Z., J. Hoffmann, M. Erdel, I. E. Eder, A. Hobisch, A. Hittmair, G. Bartsch, et al. “Switch from Antagonist to Agonist of the Androgen Receptor Blocker Bicalutamide Is Associated with Prostate Tumour Progression in a New Model System.” *British Journal of Cancer* 81, no. 2 (September 1999): 242–51. https://doi.org/10.1038/sj.bjc.6690684.

55. Tran, Chris, Samedy Ouk, Nicola J. Clegg, Yu Chen, Philip A. Watson, Vivek Arora, John Wongvipat, et al. “Development of a Second-Generation Antiandrogen for Treatment of Advanced Prostate Cancer.” *Science (New York, N.Y.)* 324, no. 5928 (May 8, 2009): 787–90. https://doi.org/10.1126/science.1168175.

56. Scher, Howard I., Karim Fizazi, Fred Saad, Mary-Ellen Taplin, Cora N. Sternberg, Kurt Miller, Ronald De Wit, et al. “Effect of MDV3100, an Androgen Receptor Signaling Inhibitor (ARSI), on Overall Survival in Patients with Prostate Cancer Postdocetaxel: Results from the Phase III AFFIRM Study.” *Journal of Clinical Oncology* 30, no. 5_suppl (February 10, 2012): LBA1–LBA1. https://doi.org/10.1200/jco.2012.30.5_suppl.lba1.

57. Beer, Tomasz M., Andrew J. Armstrong, Dana E. Rathkopf, Yohann Loriot, Cora N. Sternberg, Celestia S. Higano, Peter Iversen, et al. “Enzalutamide in Metastatic Prostate Cancer before Chemotherapy.” *New England Journal of Medicine* 371, no. 5 (July 31, 2014): 424–33. https://doi.org/10.1056/NEJMoa1405095.

58. Hussain, Maha, Karim Fizazi, Fred Saad, Per Rathenborg, Neal D. Shore, Eren Demirhan, Katharina Modelska, De Phung, Andrew Krivoshik, and Cora N. Sternberg. “PROSPER: A Phase 3, Randomized, Double-Blind, Placebo (PBO)-Controlled Study of Enzalutamide (ENZA) in Men with Nonmetastatic Castration-Resistant Prostate Cancer (M0 CRPC).” *Journal of Clinical Oncology* 36, no. 6_suppl (February 20, 2018): 3–3. https://doi.org/10.1200/JCO.2018.36.6_suppl.3.

59. Mori, Keiichiro, Hadi Mostafaei, Benjamin Pradere, Reza Sari Motlagh, Fahad Quhal, Ekaterina Laukhtina, Victor M. Schuettfort, et al. “Apalutamide, Enzalutamide, and Darolutamide for Non-gl Castration-Resistant Prostate Cancer: A Systematic Review and Network Meta-Analysis.” *International Journal of Clinical Oncology* 25, no. 11 (November 1, 2020): 1892–1900. https://doi.org/10.1007/s10147-020-01777-9.

60. Fizazi, Karim, Neal D. Shore, Teuvo Tammela, Albertas Ulys, Egils Vjaters, Sergey Polyakov, Mindaugas Jievaltas, et al. “Overall Survival (OS) Results of Phase III ARAMIS Study of Darolutamide (DARO) Added to Androgen Deprivation Therapy (ADT) for Nonmetastatic Castration-Resistant Prostate Cancer (NmCRPC).” *Journal of Clinical Oncology* 38, no. 15_suppl (May 20, 2020): 5514–5514. https://doi.org/10.1200/JCO.2020.38.15_suppl.5514.

61. Small, Eric Jay, Fred Saad, Simon Chowdhury, Boris A. Hadaschik, Julie Nicole Graff, David Olmos, Paul N. Mainwaring, et al. “SPARTAN, a Phase 3 Double-Blind, Randomized Study of Apalutamide (APA) versus Placebo (PBO) in Patients (Pts) with Nonmetastatic Castration-Resistant Prostate Cancer (NmCRPC).” *Journal of Clinical Oncology* 36, no. 6_suppl (February 20, 2018): 161–161. https://doi.org/10.1200/JCO.2018.36.6_suppl.161.

62. Gomez, Lissette, Jason R. Kovac, and Dolores J. Lamb. “CYP17A1 Inhibitors in Castration-Resistant Prostate Cancer.” *Steroids* 95 (March 1, 2015): 80–87. https://doi.org/10.1016/j.steroids.2014.12.021.

63. Montgomery, R. Bruce, Elahe A. Mostaghel, Robert Vessella, David L. Hess, Thomas F. Kalhorn, Celestia S. Higano, Lawrence D. True, and Peter S. Nelson. “Maintenance of Intratumoral Androgens in Metastatic Prostate Cancer: A Mechanism for Castration-Resistant Tumor Growth.” *Cancer Research* 68, no. 11 (June 2, 2008): 4447–54. https://doi.org/10.1158/0008-5472.CAN-08-0249.

64. Thakur, Abhimanyu, Aishwarya Roy, Arijit Ghosh, Mohit Chhabra, and Sugato Banerjee. “Abiraterone Acetate in the Treatment of Prostate Cancer.” *Biomedicine & Pharmacotherapy* 101 (May 1, 2018): 211–18. https://doi.org/10.1016/j.biopha.2018.02.067.

65. Narayanan, Ramesh, Christopher C. Coss, and James T. Dalton. “Development of Selective Androgen Receptor Modulators (SARMs).” *Molecular and Cellular Endocrinology*, Androgens – revisiting their role as pleiotropic regulators of tissue function beyond the male reproductive system, 465 (April 15, 2018): 134–42. https://doi.org/10.1016/j.mce.2017.06.013.

66. Mohler, Michael L., Arunima Sikdar, Suriyan Ponnusamy, Dong-Jin Hwang, Yali He, Duane D. Miller, and Ramesh Narayanan. “An Overview of Next-Generation Androgen Receptor-Targeted Therapeutics in Development for the Treatment of Prostate Cancer.” *International Journal of Molecular Sciences* 22, no. 4 (January 2021): 2124. https://doi.org/10.3390/ijms22042124.

67. Yu, Ziyang, Changmeng Cai, Shuai Gao, Nicholas I. Simon, Howard C. Shen, and Steven P. Balk. “Galeterone Prevents Androgen Receptor Binding to Chromatin and Enhances Degradation of Mutant Androgen Receptor.” *Clinical Cancer Research: An Official Journal of the American Association for Cancer Research* 20, no. 15 (August 1, 2014): 4075–85. https://doi.org/10.1158/1078-0432.CCR-14-0292.

68. Flanagan, John J., and Taavi K. Neklesa. “Targeting Nuclear Receptors with PROTAC Degraders.” *Molecular and Cellular Endocrinology* 493 (August 1, 2019): 110452. https://doi.org/10.1016/j.mce.2019.110452.

69. Han, Xin, Chao Wang, Chong Qin, Weiguo Xiang, Ester Fernandez-Salas, Chao-Yie Yang, Mi Wang, et al. “Discovery of ARD-69 as a Highly Potent Proteolysis Targeting Chimera (PROTAC) Degrader of Androgen Receptor (AR) for the Treatment of Prostate Cancer.” *Journal of Medicinal Chemistry* 62, no. 2 (January 24, 2019): 941–64. https://doi.org/10.1021/acs.jmedchem.8b01631.

70. Kregel, Steven, Chao Wang, Xin Han, Lanbo Xiao, Ester Fernandez-Salas, Pushpinder Bawa, Brooke L. McCollum, et al. “Androgen Receptor Degraders Overcome Common Resistance Mechanisms Developed during Prostate Cancer

Treatment." *Neoplasia* 22, no. 2 (February 1, 2020): 111–19. https://doi.org/10.1016/j.neo.2019.12.003.

71. Radaeva, Mariia, Fuqiang Ban, Fan Zhang, Eric LeBlanc, Nada Lallous, Paul S. Rennie, Martin E. Gleave, and Artem Cherkasov. "Development of Novel Inhibitors Targeting the D-Box of the DNA Binding Domain of Androgen Receptor." *International Journal of Molecular Sciences* 22, no. 5 (January 2021): 2493. https://doi.org/10.3390/ijms22052493.

72. Crawford, E. David, Mario A. Eisenberger, David G. McLeod, Joseph T. Spaulding, Ralph Benson, F. Andrew Dorr, Brent A. Blumenstein, Marilyn A. Davis, and Phyllis J. Goodman. "A Controlled Trial of Leuprolide with and without Flutamide in Prostatic Carcinoma." *New England Journal of Medicine* 321, no. 7 (August 17, 1989): 419–24. https://doi.org/10.1056/NEJM198908173210702.

73. "Maximum Androgen Blockade in Advanced Prostate Cancer: An Overview of the Randomised Trials." *The Lancet* 355, no. 9214 (April 29, 2000): 1491–98. https://doi.org/10.1016/S0140-6736(00)02163-2.

74. Davis, Ian D., Andrew J. Martin, Martin R. Stockler, Stephen Begbie, Kim N. Chi, Simon Chowdhury, Xanthi Coskinas, et al. "Enzalutamide with Standard First-Line Therapy in Metastatic Prostate Cancer." *New England Journal of Medicine* 381, no. 2 (July 11, 2019): 121–31. https://doi.org/10.1056/NEJMoa1903835.

75. Armstrong, Andrew J., Russell Zelig Szmulewitz, Daniel Peter Petrylak, Arnauld Villers, Arun Azad, Antonio Alcaraz, Boris Y. Alekseev, et al. "Phase III Study of Androgen Deprivation Therapy (ADT) with Enzalutamide (ENZA) or Placebo (PBO) in Metastatic Hormone-Sensitive Prostate Cancer (MHSPC): The ARCHES Trial." *Journal of Clinical Oncology* 37, no. 7_suppl (March 2019): 687–687. https://doi.org/10.1200/JCO.2019.37.7_suppl.687.

76. Fizazi, Karim, Namphuong Tran, Luis Enrique Fein, Nobuaki Matsubara, Alfredo Rodríguez Antolín, Boris Y. Alekseev, Mustafa Ozguroglu, et al. "LATITUDE: A Phase III, Double-Blind, Randomized Trial of Androgen Deprivation Therapy with Abiraterone Acetate plus Prednisone or Placebos in Newly Diagnosed High-Risk Metastatic Hormone-Naive Prostate Cancer." *Journal of Clinical Oncology* 35, no. 18_suppl (June 20, 2017): LBA3–LBA3. https://doi.org/10.1200/JCO.2017.35.18_suppl.LBA3.

77. Buck, Stefan A. J., Stijn L. W. Koolen, Ron H. J. Mathijssen, Ronald de Wit, and Robert J. van Soest. "Cross-Resistance and Drug Sequence in Prostate Cancer." *Drug Resistance Updates* 56 (May 1, 2021): 100761. https://doi.org/10.1016/j.drup.2021.100761.

78. Mori, Keiichiro, Noriyoshi Miura, Hadi Mostafaei, Fahad Quhal, Reza Sari Motlagh, Benjamin Pradere, Shoji Kimura, et al. "Sequential Therapy of Abiraterone and Enzalutamide in Castration-Resistant Prostate Cancer: A

Systematic Review and Meta-Analysis." *Prostate Cancer and Prostatic Diseases* 23, no. 4 (December 2020): 539–48. https://doi.org/10.1038/s41391-020-0222-6.

79. Tannock, Ian F., Ronald de Wit, William R. Berry, Jozsef Horti, Anna Pluzanska, Kim N. Chi, Stephane Oudard, et al. "Docetaxel plus Prednisone or Mitoxantrone plus Prednisone for Advanced Prostate Cancer." *New England Journal of Medicine* 351, no. 15 (October 7, 2004): 1502–12. https://doi.org/10.1056/NEJMoa040720.

80. Bono, Johann de, Joaquin Mateo, Karim Fizazi, Fred Saad, Neal Shore, Shahneen Sandhu, Kim N. Chi, et al. "Olaparib for Metastatic Castration-Resistant Prostate Cancer." *New England Journal of Medicine* 382, no. 22 (May 28, 2020): 2091–2102. https://doi.org/10.1056/NEJMoa1911440.

81. Zafeiriou, Zafeiris, Diletta Bianchini, Robert Chandler, Pasquale Rescigno, Wei Yuan, Suzanne Carreira, Maialen Barrero, et al. "Genomic Analysis of Three Metastatic Prostate Cancer Patients with Exceptional Responses to Carboplatin Indicating Different Types of DNA Repair Deficiency." *European Urology* 75, no. 1 (January 1, 2019): 184–92. https://doi.org/10.1016/j.eururo.2018.09.048.

82. Stultz, Jacob, and Lawrence Fong. "How to Turn up the Heat on the Cold Immune Microenvironment of Metastatic Prostate Cancer." *Prostate Cancer and Prostatic Diseases* 24, no. 3 (September 2021): 697–717. https://doi.org/10.1038/s41391-021-00340-5.

83. Martínez-Pérez, Elizabeth, Miguel Angel Molina-Vila, and Cristina Marino-Buslje. "Panels and Models for Accurate Prediction of Tumor Mutation Burden in Tumor Samples." *Npj Precision Oncology* 5, no. 1 (April 13, 2021): 1–8. https://doi.org/10.1038/s41698-021-00169-0.

84. Kwon, Eugene D, Charles G Drake, Howard I Scher, Karim Fizazi, Alberto Bossi, Alfons J M van den Eertwegh, Michael Krainer, 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." *The Lancet Oncology* 15, no. 7 (June 1, 2014): 700–712. https://doi.org/10.1016/S1470-2045(14)70189-5.

85. Kantoff, Philip W., Celestia S. Higano, Neal D. Shore, E. Roy Berger, Eric J. Small, David F. Penson, Charles H. Redfern, et al. "Sipuleucel-T Immunotherapy for Castration-Resistant Prostate Cancer." *New England Journal of Medicine* 363, no. 5 (July 29, 2010): 411–22. https://doi.org/10.1056/NEJMoa1001294.

86. Saraç, Hilal, Artem Cherkasov, and Nathan A. Lack. "Chapter 2 - Development of Novel Androgen Receptor Inhibitors to Overcome Castrate-Resistant Prostate Cancer." In *Biological Mechanisms and the Advancing Approaches to Overcoming Cancer Drug Resistance*, edited by Andrew Freywald and Franco J. Vizeacoumar, 12:23–46. Cancer Sensitizing Agents for Chemotherapy. Academic Press, 2021. https://doi.org/10.1016/B978-0-12-821310-0.00007-3.

87. Ribeiro, Ralff C. J., M.D, Peter J. Kushner Ph.D, and John D. Baxter M.D. “The Nuclear Hormone Receptor Gene Superfamily.” *Annual Review of Medicine* 46, no. 1 (1995): 443–53. https://doi.org/10.1146/annurev.med.46.1.443.

88. Katzenellenbogen, John A., and Benita S. Katzenellenbogen. “Nuclear Hormone Receptors: Ligand-Activated Regulators of Transcription and Diverse Cell Responses.” *Chemistry & Biology* 3, no. 7 (July 1, 1996): 529–36. https://doi.org/10.1016/S1074-5521(96)90143-X.

89. Pratt, William B. “Control of Steroid Receptor Function and Cytoplasmic-Nuclear Transport by Heat Shock Proteins.” *BioEssays* 14, no. 12 (1992): 841–48. https://doi.org/10.1002/bies.950141209.

90. Carson-Jurica, Mary Anne, William T. Schrader, and Bert W. O’Malley. “Steroid Receptor Family: Structure and Functions.” *Endocrine Reviews* 11, no. 2 (May 1, 1990): 201–20. https://doi.org/10.1210/edrv-11-2-201.

91. Stashi, Erin, Brian York, and Bert W. O’Malley. “Steroid Receptor Coactivators: Servants and Masters for Control of Systems Metabolism.” *Trends in Endocrinology & Metabolism* 25, no. 7 (July 1, 2014): 337–47. https://doi.org/10.1016/j.tem.2014.05.004.

92. Gelmann, Edward P. “Molecular Biology of the Androgen Receptor.” *Journal of Clinical Oncology: Official Journal of the American Society of Clinical Oncology* 20, no. 13 (July 1, 2002): 3001–15. https://doi.org/10.1200/JCO.2002.10.018.

93. Burnstein, Kerry L. “Regulation of Androgen Receptor Levels: Implications for Prostate Cancer Progression and Therapy.” *Journal of Cellular Biochemistry* 95, no. 4 (2005): 657–69. https://doi.org/10.1002/jcb.20460.

94. Cao, Subing, Yang Zhan, and Yan Dong. “Emerging Data on Androgen Receptor Splice Variants in Prostate Cancer.” *Endocrine-Related Cancer* 23, no. 12 (December 2016): T199–210. https://doi.org/10.1530/ERC-16-0298.

95. Dehm, Scott M., and Donald J. Tindall. “Alternatively Spliced Androgen Receptor Variants.” *Endocrine-Related Cancer* 18, no. 5 (October 2011): R183–96. https://doi.org/10.1530/ERC-11-0141.

96. Tepper, Clifford G., David L. Boucher, Philip E. Ryan, Ai-Hong Ma, Liang Xia, Li-Fen Lee, Thomas G. Pretlow, and Hsing-Jien Kung. “Characterization of a Novel Androgen Receptor Mutation in a Relapsed CWR22 Prostate Cancer Xenograft and Cell Line1.” *Cancer Research* 62, no. 22 (November 15, 2002): 6606–14.

97. Dehm, Scott M., Lucy J. Schmidt, Hannelore V. Heemers, Robert L. Vessella, and Donald J. Tindall. “Splicing of a Novel Androgen Receptor Exon Generates a Constitutively Active Androgen Receptor That Mediates Prostate Cancer Therapy

Resistance." *Cancer Research* 68, no. 13 (July 1, 2008): 5469–77. https://doi.org/10.1158/0008-5472.CAN-08-0594.

98. Hu, Rong, Thomas A. Dunn, Shuanzeng Wei, Sumit Isharwal, Robert W. Veltri, Elizabeth Humphreys, Misop Han, et al. "Ligand-Independent Androgen Receptor Variants Derived from Splicing of Cryptic Exons Signify Hormone-Refractory Prostate Cancer." *Cancer Research* 69, no. 1 (December 31, 2008): 16–22. https://doi.org/10.1158/0008-5472.CAN-08-2764.

99. Hu, Rong, William B. Isaacs, and Jun Luo. "A Snapshot of the Expression Signature of Androgen Receptor Splicing Variants and Their Distinctive Transcriptional Activities." *The Prostate* 71, no. 15 (November 2011): 1656–67. https://doi.org/10.1002/pros.21382.

100. Xu, Duo, Yang Zhan, Yanfeng Qi, Bo Cao, Shanshan Bai, Wei Xu, Sanjiv S. Gambhir, et al. "Androgen Receptor Splice Variants Dimerize to Transactivate Target Genes." *Cancer Research* 75, no. 17 (September 1, 2015): 3663–71. https://doi.org/10.1158/0008-5472.CAN-15-0381.

101. Kadmiel, Mahita, and John A. Cidlowski. "Glucocorticoid Receptor Signaling in Health and Disease." *Trends in Pharmacological Sciences* 34, no. 9 (September 1, 2013): 518–30. https://doi.org/10.1016/j.tips.2013.07.003.

102. King, Heather A., Kevin W. Trotter, and Trevor K. Archer. 2012. "Chromatin Remodeling during Glucocorticoid Receptor Regulated Transactivation." *Biochimica et Biophysica Acta (BBA) - Gene Regulatory Mechanisms*, Chromatin in time and space, 1819 (7): 716–26. https://doi.org/10.1016/j.bbagrm.2012.02.019.

103. Yamada, Yasutaka, and Himisha Beltran. "Clinical and Biological Features of Neuroendocrine Prostate Cancer." *Current Oncology Reports* 23, no. 2 (January 12, 2021): 15. https://doi.org/10.1007/s11912-020-01003-9.

104. Beltran, Himisha, David S. Rickman, Kyung Park, Sung Suk Chae, Andrea Sboner, Theresa Y. MacDonald, Yuwei Wang, et al. "Molecular Characterization of Neuroendocrine Prostate Cancer and Identification of New Drug Targets." *Cancer Discovery* 1, no. 6 (November 16, 2011): 487–95. https://doi.org/10.1158/2159-8290.CD-11-0130.

105. Watson, Philip A., Vivek K. Arora, and Charles L. Sawyers. "Emerging Mechanisms of Resistance to Androgen Receptor Inhibitors in Prostate Cancer." *Nature Reviews. Cancer* 15, no. 12 (December 2015): 701–11. https://doi.org/10.1038/nrc4016.

106. Horoszewicz, J. S., S. S. Leong, E. Kawinski, J. P. Karr, H. Rosenthal, T. M. Chu, E. A. Mirand, and G. P. Murphy. "LNCaP Model of Human Prostatic Carcinoma." *Cancer Research* 43, no. 4 (April 1983): 1809–18.

107. Veldscholte, J., C. Ris-Stalpers, G. G. Kuiper, G. Jenster, C. Berrevoets, E. Claassen, H. C. van Rooij, J. Trapman, A. O. Brinkmann, and E. Mulder. "A Mutation in the Ligand Binding Domain of the Androgen Receptor of Human LNCaP Cells Affects Steroid Binding Characteristics and Response to Anti-Androgens." *Biochemical and Biophysical Research Communications* 173, no. 2 (December 14, 1990): 534–40. https://doi.org/10.1016/s0006-291x(05)80067-1.

108. Schuurmans, A. L. G., J. Bolt, J. Veldscholte, and E. Mulder. "Regulation of Growth of LNCaP Human Prostate Tumor Cells by Growth Factors and Steroid Hormones." *The Journal of Steroid Biochemistry and Molecular Biology* 40, no. 1 (January 1, 1991): 193–97. https://doi.org/10.1016/0960-0760(91)90182-5.

109. Suzuki, H., N. Sato, Y. Watabe, M. Masai, S. Seino, and J. Shimazaki. "Androgen Receptor Gene Mutations in Human Prostate Cancer." *The Journal of Steroid Biochemistry and Molecular Biology* 46, no. 6 (December 1993): 759–65. https://doi.org/10.1016/0960-0760(93)90316-o.

110. Visakorpi, T., E. Hyytinen, P. Koivisto, M. Tanner, R. Keinänen, C. Palmberg, A. Palotie, T. Tammela, J. Isola, and O. P. Kallioniemi. "In Vivo Amplification of the Androgen Receptor Gene and Progression of Human Prostate Cancer." *Nature Genetics* 9, no. 4 (April 1995): 401–6. https://doi.org/10.1038/ng0495-401.

111. Gregory, Christopher W., Bin He, Raymond T. Johnson, O. Harris Ford, James L. Mohler, Frank S. French, and Elizabeth M. Wilson. "A Mechanism for Androgen Receptor-Mediated Prostate Cancer Recurrence after Androgen Deprivation Therapy1." *Cancer Research* 61, no. 11 (June 1, 2001): 4315–19.

112. Chen, Charlie D., Derek S. Welsbie, Chris Tran, Sung Hee Baek, Randy Chen, Robert Vessella, Michael G. Rosenfeld, and Charles L. Sawyers. "Molecular Determinants of Resistance to Antiandrogen Therapy." *Nature Medicine* 10, no. 1 (January 2004): 33–39. https://doi.org/10.1038/nm972.

113. Grasso, Catherine S., Yi-Mi Wu, Dan R. Robinson, Xuhong Cao, Saravana M. Dhanasekaran, Amjad P. Khan, Michael J. Quist, et al. "The Mutational Landscape of Lethal Castrate Resistant Prostate Cancer." *Nature* 487, no. 7406 (July 12, 2012): 239–43. https://doi.org/10.1038/nature11125.

114. Beltran, Himisha, Roman Yelensky, Garrett M. Frampton, Kyung Park, Sean R. Downing, Theresa Y. MacDonald, Mirna Jarosz, et al. "Targeted Next-Generation Sequencing of Advanced Prostate Cancer Identifies Potential Therapeutic Targets and Disease Heterogeneity." *European Urology* 63, no. 5 (May 2013): 920–26. https://doi.org/10.1016/j.eururo.2012.08.053.

115. Bohl, Casey E., Wenqing Gao, Duane D. Miller, Charles E. Bell, and James T. Dalton. "Structural Basis for Antagonism and Resistance of Bicalutamide in Prostate Cancer." *Proceedings of the National Academy of Sciences* 102, no. 17 (April 26, 2005): 6201–6. https://doi.org/10.1073/pnas.0500381102.

116. Korpal, Manav, Joshua M. Korn, Xueliang Gao, Daniel P. Rakiec, David A. Ruddy, Shivang Doshi, Jing Yuan, et al. "An F876L Mutation in Androgen Receptor Confers Genetic and Phenotypic Resistance to MDV3100 (Enzalutamide)." *Cancer Discovery* 3, no. 9 (September 9, 2013): 1030–43. https://doi.org/10.1158/2159-8290.CD-13-0142.

117. Eisermann, Kurtis, Dan Wang, Yifeng Jing, Laura E. Pascal, and Zhou Wang. "Androgen Receptor Gene Mutation, Rearrangement, Polymorphism." *Translational Andrology and Urology* 2, no. 3 (September 2013): 137–47. https://doi.org/10.3978/j.issn.2223-4683.2013.09.15.

118. Steinkamp, Mara P., Orla A. O'Mahony, Michele Brogley, Haniya Rehman, Elizabeth W. LaPensee, Saravana Dhanasekaran, Matthias D. Hofer, et al. "TREATMENT-DEPENDENT ANDROGEN RECEPTOR MUTATIONS IN PROSTATE CANCER EXPLOIT MULTIPLE MECHANISMS TO EVADE THERAPY." *Cancer Research* 69, no. 10 (May 15, 2009): 4434–42. https://doi.org/10.1158/0008-5472.CAN-08-3605.

119. Monge, A., M. Jagla, G. Lapouge, S. Sasorith, M. Cruchant, J.-M. Wurtz, D. Jacqmin, J.-P. Bergerat, and J. Céraline. "Unfaithfulness and Promiscuity of a Mutant Androgen Receptor in a Hormone-Refractory Prostate Cancer." *Cellular and Molecular Life Sciences CMLS* 63, no. 4 (February 1, 2006): 487–97. https://doi.org/10.1007/s00018-005-5471-y.

120. Titus, Mark A., Michael J. Schell, Fred B. Lih, Kenneth B. Tomer, and James L. Mohler. "Testosterone and Dihydrotestosterone Tissue Levels in Recurrent Prostate Cancer." *Clinical Cancer Research* 11, no. 13 (July 6, 2005): 4653–57. https://doi.org/10.1158/1078-0432.CCR-05-0525.

121. Chang, Kai-Hsiung, Rui Li, Mahboubeh Papari-Zareei, Lori Watumull, Yan Daniel Zhao, Richard J. Auchus, and Nima Sharifi. "Dihydrotestosterone Synthesis Bypasses Testosterone to Drive Castration-Resistant Prostate Cancer." *Proceedings of the National Academy of Sciences* 108, no. 33 (August 16, 2011): 13728–33. https://doi.org/10.1073/pnas.1107898108.

122. Miller, Walter L., and Richard J. Auchus. "The 'Backdoor Pathway' of Androgen Synthesis in Human Male Sexual Development." *PLOS Biology* 17, no. 4 (April 3, 2019): e3000198. https://doi.org/10.1371/journal.pbio.3000198.

123. Logothetis, Christopher J., Eleni Efstathiou, Fil Manuguid, and Peter Kirkpatrick. "Abiraterone Acetate." *Nature Reviews Drug Discovery* 10, no. 8 (August 1, 2011): 573–74. https://doi.org/10.1038/nrd3516.

124. Guo, Zhiyong, Xi Yang, Feng Sun, Richeng Jiang, Douglas E. Linn, Hege Chen, Hegang Chen, et al. "A Novel Androgen Receptor Splice Variant Is Upregulated during Prostate Cancer Progression and Promotes Androgen-Depletion-Resistant Growth." *Cancer Research* 69, no. 6 (March 15, 2009): 2305–13. https://doi.org/10.1158/0008-5472.CAN-08-3795.

125. Cao, Bo, Yanfeng Qi, Guanyi Zhang, Duo Xu, Yang Zhan, Xavier Alvarez, Zhiyong Guo, et al. "Androgen Receptor Splice Variants Activating the Full-Length Receptor in Mediating Resistance to Androgen-Directed Therapy." *Oncotarget* 5, no. 6 (March 4, 2014): 1646–56.

126. Li, Yingming, Siu Chiu Chan, Lucas J. Brand, Tae Hyun Hwang, Kevin A. T. Silverstein, and Scott M. Dehm. "Androgen Receptor Splice Variants Mediate Enzalutamide Resistance in Castration-Resistant Prostate Cancer Cell Lines." *Cancer Research* 73, no. 2 (January 15, 2013): 483–89. https://doi.org/10.1158/0008-5472.CAN-12-3630.

127. Yamamoto, Yoshiaki, Yohann Loriot, Eliana Beraldi, Fan Zhang, Alexander W. Wyatt, Nader Al Nakouzi, Fan Mo, et al. "Generation 2.5 Antisense Oligonucleotides Targeting the Androgen Receptor and Its Splice Variants Suppress Enzalutamide-Resistant Prostate Cancer Cell Growth." *Clinical Cancer Research: An Official Journal of the American Association for Cancer Research* 21, no. 7 (April 1, 2015): 1675–87. https://doi.org/10.1158/1078-0432.CCR-14-1108.

128. Hu, Rong, Changxue Lu, Elahe A. Mostaghel, Srinivasan Yegnasubramanian, Meltem Gurel, Clare Tannahill, Joanne Edwards, et al. "Distinct Transcriptional Programs Mediated by the Ligand-Dependent Full-Length Androgen Receptor and Its Splice Variants in Castration-Resistant Prostate Cancer." *Cancer Research* 72, no. 14 (July 15, 2012): 3457–62. https://doi.org/10.1158/0008-5472.CAN-11-3892.

129. Cato, Laura, Jonas de Tribolet-Hardy, Irene Lee, Jaice T. Rottenberg, Ilsa Coleman, Diana Melchers, Rene Houtman, et al. "ARv7 Represses Tumor-Suppressor Genes in Castration-Resistant Prostate Cancer." *Cancer Cell* 35, no. 3 (March 18, 2019): 401-413.e6. https://doi.org/10.1016/j.ccell.2019.01.008.

130. Chen, Zhong, Dayong Wu, Jennifer M. Thomas-Ahner, Changxue Lu, Pei Zhao, Qingfu Zhang, Connor Geraghty, et al. "Diverse AR-V7 Cistromes in Castration-Resistant Prostate Cancer Are Governed by HoxB13." *Proceedings of the National Academy of Sciences of the United States of America* 115, no. 26 (June 26, 2018): 6810–15. https://doi.org/10.1073/pnas.1718811115.

131. Sugiura, Masahiro, Hiroaki Sato, Atsushi Okabe, Masaki Fukuyo, Yasunobu Mano, Ken-ichi Shinohara, Bahityar Rahmutulla, et al. "Identification of AR-V7 Downstream Genes Commonly Targeted by AR/AR-V7 and Specifically Targeted by AR-V7 in Castration Resistant Prostate Cancer." *Translational Oncology* 14, no. 1 (January 1, 2021): 100915. https://doi.org/10.1016/j.tranon.2020.100915.

132. Liang, Jiaqian, Liyang Wang, Larysa Poluben, Mannan Nouri, Seiji Arai, Lisha Xie, Olga S. Voznesensky, et al. "Androgen Receptor Splice Variant 7 Functions Independently of the Full Length Receptor in Prostate Cancer Cells." *Cancer*

Letters 519 (October 28, 2021): 172–84. https://doi.org/10.1016/j.canlet.2021.07.013.

133. Basil, Paul, Matthew J. Robertson, William E. Bingman, Amit K. Dash, William C. Krause, Ayesha A. Shafi, Badrajee Piyarathna, Cristian Coarfa, and Nancy L. Weigel. "Cistrome and Transcriptome Analysis Identifies Unique Androgen Receptor (AR) and AR-V7 Splice Variant Chromatin Binding and Transcriptional Activities." *Scientific Reports* 12, no. 1 (March 30, 2022): 5351. https://doi.org/10.1038/s41598-022-09371-x.

134. Antonarakis, Emmanuel S., Changxue Lu, Hao Wang, Brandon Luber, Mary Nakazawa, Jeffrey C. Roeser, Yan Chen, et al. "AR-V7 and Resistance to Enzalutamide and Abiraterone in Prostate Cancer." *New England Journal of Medicine* 371, no. 11 (September 11, 2014): 1028–38. https://doi.org/10.1056/NEJMoa1315815.

135. Qu, Fangfang, Wanling Xie, Mari Nakabayashi, Haitao Zhang, Seong Ho Jeong, Xiaodong Wang, Kazumasa Komura, et al. "Association of AR-V7 and Prostate-Specific Antigen RNA Levels in Blood with Efficacy of Abiraterone Acetate and Enzalutamide Treatment in Men with Prostate Cancer." *Clinical Cancer Research* 23, no. 3 (January 31, 2017): 726–34. https://doi.org/10.1158/1078-0432.CCR-16-1070.

136. Kumar, Raj. "Emerging Role of Glucocorticoid Receptor in Castration Resistant Prostate Cancer: A Potential Therapeutic Target." *Journal of Cancer* 11, no. 3 (January 1, 2020): 696–701. https://doi.org/10.7150/jca.32497.

137. Smith, R. G., A. J. Syms, A. Nag, S. Lerner, and J. S. Norris. "Mechanism of the Glucocorticoid Regulation of Growth of the Androgen-Sensitive Prostate-Derived R3327H-G8-A1 Tumor Cell Line." *Journal of Biological Chemistry* 260, no. 23 (October 15, 1985): 12454–63. https://doi.org/10.1016/S0021-9258(17)38894-4.

138. Nishimura, Kazuo, Norio Nonomura, Eiichi Satoh, Yasunori Harada, Masashi Nakayama, Takashi Tokizane, Tatsunari Fukui, et al. "Potential Mechanism for the Effects of Dexamethasone on Growth of Androgen-Independent Prostate Cancer." *JNCI: Journal of the National Cancer Institute* 93, no. 22 (November 21, 2001): 1739–46. https://doi.org/10.1093/jnci/93.22.1739.

139. Szmulewitz, Russell Z., Elizabeth Chung, Hikmat Al-Ahmadie, Silver Daniel, Masha Kocherginsky, Aria Razmaria, Gregory P. Zagaja, Charles B. Brendler, Walter M. Stadler, and Suzanne D. Conzen. "Serum/Glucocorticoid-Regulated Kinase 1 Expression in Primary Human Prostate Cancers." *The Prostate* 72, no. 2 (2012): 157–64. https://doi.org/10.1002/pros.21416.

140. Isikbay, Masis, Kristen Otto, Steven Kregel, Jacob Kach, Yi Cai, Donald J. Vander Griend, Suzanne D. Conzen, and Russell Z. Szmulewitz. "Glucocorticoid Receptor Activity Contributes to Resistance to Androgen-Targeted Therapy in Prostate

Cancer." *Hormones and Cancer* 5, no. 2 (April 1, 2014): 72–89. https://doi.org/10.1007/s12672-014-0173-2.

141. Arora, Vivek K., Emily Schenkein, Rajmohan Murali, Sumit K. Subudhi, John Wongvipat, Minna D. Balbas, Neel Shah, et al. 2013. "Glucocorticoid Receptor Confers Resistance to Antiandrogens by Bypassing Androgen Receptor Blockade." *Cell* 155 (6): 1309–22. https://doi.org/10.1016/j.cell.2013.11.012.

142. Long, Tiha M., Eva Y. Tonsing-Carter, Wen-Ching Chan, Donald Vander Griend, Suzanne D. Conzen, and Russell Z. Szmulewitz. 2018. "Abstract 944: Glucocorticoid Receptor (GR)-Mediated Activation of Cyclic-Adenosine Monophosphate (CAMP) Pathway Gene Expression Following Androgen Receptor (AR) Antagonism of Prostate Cancer." *Cancer Research* 78 (13_Supplement): 944. https://doi.org/10.1158/1538-7445.AM2018-944.

143. Lin, Ming-Fong, Mark H. Kawachi, Fen-Fen Lin, Michael R. Stallcup, and Steven M. Grunberg. 1995. "Growth Inhibition of Androgen-Insensitive Human Prostate Carcinoma Cells by a 19-Norsteroid Derivative Agent, Mifepristone." *The Prostate* 26 (4): 194–204. https://doi.org/10.1002/pros.2990260405.

144. Tieszen, Chelsea R., Alicia A. Goyeneche, BreeAnn N. Brandhagen, Casey T. Ortbahn, and Carlos M. Telleria. 2011. "Antiprogestin Mifepristone Inhibits the Growth of Cancer Cells of Reproductive and Non-Reproductive Origin Regardless of Progesterone Receptor Expression." *BMC Cancer* 11 (1): 207. https://doi.org/10.1186/1471-2407-11-207.

145. Puhr, Martin, Julia Hoefer, Andrea Eigentler, Christian Ploner, Florian Handle, Georg Schaefer, Jan Kroon, et al. 2018. "The Glucocorticoid Receptor Is a Key Player for Prostate Cancer Cell Survival and a Target for Improved Antiandrogen Therapy." *Clinical Cancer Research* 24 (4): 927–38. https://doi.org/10.1158/1078-0432.CCR-17-0989.

146. Taplin, Mary-Ellen, Judith Manola, William K. Oh, Philip W. Kantoff, Glenn J. Bubley, Matthew Smith, Diana Barb, Christos Mantzoros, Edward P. Gelmann, and Steven P. Balk. 2008. "A Phase II Study of Mifepristone (RU-486) in Castration-Resistant Prostate Cancer, with a Correlative Assessment of Androgen-Related Hormones." *BJU International* 101 (9): 1084–89. https://doi.org/10.1111/j.1464-410X.2008.07509.x.

147. Kach, Jacob, Tiha M. Long, Phillip Selman, Eva Y. Tonsing-Carter, Maria A. Bacalao, Ricardo R. Lastra, Larischa de Wet, et al. 2017. "Selective Glucocorticoid Receptor Modulators (SGRMs) Delay Castrate-Resistant Prostate Cancer Growth." *Molecular Cancer Therapeutics* 16 (8): 1680–92. https://doi.org/10.1158/1535-7163.MCT-16-0923.

148. Sharp, Adam, Ilsa Coleman, Wei Yuan, Cynthia Sprenger, David Dolling, Daniel Nava Rodrigues, Joshua W. Russo, et al. 2019. "Androgen Receptor Splice Variant-

7 Expression Emerges with Castration Resistance in Prostate Cancer." *The Journal of Clinical Investigation* 129 (1): 192–208. https://doi.org/10.1172/JCI122819.

149. Shah, Neel, Ping Wang, John Wongvipat, Wouter R Karthaus, Wassim Abida, Joshua Armenia, Shira Rockowitz, et al. 2017. "Regulation of the Glucocorticoid Receptor via a BET-Dependent Enhancer Drives Antiandrogen Resistance in Prostate Cancer." Edited by Maarten van Lohuizen. *ELife* 6 (September): e27861. https://doi.org/10.7554/eLife.27861.

150. Machleidt, Thomas, Carolyn C. Woodroofe, Marie K. Schwinn, Jacqui Méndez, Matthew B. Robers, Kris Zimmerman, Paul Otto, Danette L. Daniels, Thomas A. Kirkland, and Keith V. Wood. 2015. "NanoBRET—A Novel BRET Platform for the Analysis of Protein–Protein Interactions." *ACS Chemical Biology* 10 (8): 1797–1804. https://doi.org/10.1021/acschembio.5b00143.

151. Dalal, Kush, Fuqiang Ban, Huifang Li, Hélène Morin, Mani Roshan-Moniri, Kevin J. Tam, Ashley Shepherd, et al. 2018. "Selectively Targeting the Dimerization Interface of Human Androgen Receptor with Small-Molecules to Treat Castration-Resistant Prostate Cancer." *Cancer Letters* 437 (November): 35–43. https://doi.org/10.1016/j.canlet.2018.08.016.

152. Royen, Martin E. van, Wiggert A. van Cappellen, Carola de Vos, Adriaan B. Houtsmuller, and Jan Trapman. 2012. "Stepwise Androgen Receptor Dimerization." *Journal of Cell Science* 125 (8): 1970–79. https://doi.org/10.1242/jcs.096792.

153. Roggero, Carlos M., Lianjin Jin, Subing Cao, Rajni Sonavane, Noa G. Kopplin, Huy Q. Ta, Dede N. Ekoue, et al. 2021. "A Detailed Characterization of Stepwise Activation of the Androgen Receptor Variant 7 in Prostate Cancer Cells." *Oncogene* 40 (6): 1106–17. https://doi.org/10.1038/s41388-020-01585-5.

154. Sramkoski, R. Michael, Thomas G. Pretlow, Joseph M. Giaconia, Theresa P. Pretlow, Stuart Schwartz, Man-Sun Sy, Susan R. Marengo, Johng S. Rhim, Desheng Zhang, and James W. Jacobberger. 1999. "A New Human Prostate Carcinoma Cell Line, 22Rv1." *In Vitro Cellular & Developmental Biology - Animal* 35 (7): 403–9. https://doi.org/10.1007/s11626-999-0115-4.

155. Korenchuk, S, J E Lehr, L MClean, Y G Lee, S Whitney, R Vessella, D L Lin, and K J Pienta. 2001. "VCaP, a Cell-Based Model System of Human Prostate Cancer." *In Vivo (Athens, Greece)* 15 (2): 163–68.

156. Van Etten, Jamie L., Michael Nyquist, Yingming Li, Rendong Yang, Yeung Ho, Rachel Johnson, Olivia Ondigi, Daniel F. Voytas, Christine Henzler, and Scott M. Dehm. 2017. "Targeting a Single Alternative Polyadenylation Site Coordinately Blocks Expression of Androgen Receptor MRNA Splice Variants in Prostate Cancer." *Cancer Research* 77 (19): 5228–35. https://doi.org/10.1158/0008-5472.CAN-17-0320.

157. Henzler, Christine, Yingming Li, Rendong Yang, Terri McBride, Yeung Ho, Cynthia Sprenger, Gang Liu, et al. 2016. “Truncation and Constitutive Activation of the Androgen Receptor by Diverse Genomic Rearrangements in Prostate Cancer.” *Nature Communications* 7 (1): 13668. https://doi.org/10.1038/ncomms13668.

158. Watson, Philip A., Yinan F. Chen, Minna D. Balbas, John Wongvipat, Nicholas D. Socci, Agnes Viale, Kwanghee Kim, and Charles L. Sawyers. 2010. “Constitutively Active Androgen Receptor Splice Variants Expressed in Castration-Resistant Prostate Cancer Require Full-Length Androgen Receptor.” *Proceedings of the National Academy of Sciences* 107 (39): 16759–65. https://doi.org/10.1073/pnas.1012443107.

159. Bonkhoff, Helmut, Thomas Fixemer, Isabel Hunsicker, and Klaus Remberger. 2001. “Progesterone Receptor Expression in Human Prostate Cancer: Correlation with Tumor Progression.” *The Prostate* 48 (4): 285–91. https://doi.org/10.1002/pros.1108.

160. Mukherji, Deborah, Carmel J. Pezaro, and Johann S. De-Bono. 2012. “MDV3100 for the Treatment of Prostate Cancer.” *Expert Opinion on Investigational Drugs* 21 (2): 227–33. https://doi.org/10.1517/13543784.2012.651125.

161. Richards, Mark W., Selena G. Burgess, Evon Poon, Anne Carstensen, Martin Eilers, Louis Chesler, and Richard Bayliss. 2016. “Structural Basis of N-Myc Binding by Aurora-A and Its Destabilization by Kinase Inhibitors.” *Proceedings of the National Academy of Sciences* 113 (48): 13726–31. https://doi.org/10.1073/pnas.1610626113.

162. Beltran, Himisha, Clara Oromendia, Daniel C. Danila, Bruce Montgomery, Christopher Hoimes, Russell Z. Szmulewitz, Ulka Vaishampayan, et al. 2019. “A Phase II Trial of the Aurora Kinase A Inhibitor Alisertib for Patients with Castration-Resistant and Neuroendocrine Prostate Cancer: Efficacy and Biomarkers.” *Clinical Cancer Research* 25 (1): 43–51. https://doi.org/10.1158/1078-0432.CCR-18-1912.

163. Ban, Fuqiang, Kush Dalal, Huifang Li, Eric LeBlanc, Paul S. Rennie, and Artem Cherkasov. 2017. “Best Practices of Computer-Aided Drug Discovery: Lessons Learned from the Development of a Preclinical Candidate for Prostate Cancer with a New Mechanism of Action.” *Journal of Chemical Information and Modeling* 57 (5): 1018–28. https://doi.org/10.1021/acs.jcim.7b00137.

164. Hu, Taishan, Nilesh Chitnis, Dimitri Monos, and Anh Dinh. 2021. “Next-Generation Sequencing Technologies: An Overview.” *Human Immunology*, Next Generation Sequencing and its Application to Medical Laboratory Immunology, 82 (11): 801–11. https://doi.org/10.1016/j.humimm.2021.02.012.

165. Ki, Chang-Seok. 2021. “Recent Advances in the Clinical Application of Next-Generation Sequencing.” *Pediatric Gastroenterology, Hepatology & Nutrition* 24 (1): 1–6. https://doi.org/10.5223/pghn.2021.24.1.1.

166. Lowe, Derek. “Trouble at Santa Cruz Biotechnology.” https://www.science.org/content/blog-post/trouble-santa-cruz-biotechnology.

167. Montanera, Kaitlin N., Zobia Anwar, Sherin M. Shibin, and Ho Sung Rhee. 2020. “Chapter Sixteen - ChIP-Exo: A Method to Study Chromatin Structure and Organization at near-Nucleotide Resolution.” In *Epigenetics Methods*, edited by Trygve Tollefsbol, 18:323–52. Translational Epigenetics. Academic Press. https://doi.org/10.1016/B978-0-12-819414-0.00016-1.

168. Hainer, Sarah J., and Thomas G. Fazzio. 2019. “High-Resolution Chromatin Profiling Using CUT&RUN.” *Current Protocols in Molecular Biology* 126 (1): e85. https://doi.org/10.1002/cpmb.85.

169. Markowski, Mark C., John L. Silberstein, James R. Eshleman, Mario A. Eisenberger, Jun Luo, and Emmanuel S. Antonarakis. 2017. “Clinical Utility of CLIA-Grade AR-V7 Testing in Patients With Metastatic Castration-Resistant Prostate Cancer.” *JCO Precision Oncology*, no. 1 (November): 1–9. https://doi.org/10.1200/PO.17.00127.

170. Streicher, Justin, Brian Lee Meyerson, Vidhya Karivedu, and Abhinav Sidana. 2019. “A Review of Optimal Prostate Biopsy: Indications and Techniques.” *Therapeutic Advances in Urology* 11 (January): 1756287219870074. https://doi.org/10.1177/1756287219870074.

171. Lu, Yi-Tsung, Kevin Delijani, Andrew Mecum, and Amir Goldkorn. 2019. “Current Status of Liquid Biopsies for the Detection and Management of Prostate Cancer.” *Cancer Management and Research* 11 (June): 5271–91. https://doi.org/10.2147/CMAR.S170380.

172. Ewing, Charles M., Anna M. Ray, Ethan M. Lange, Kimberly A. Zuhlke, Christiane M. Robbins, Waibhav D. Tembe, Kathleen E. Wiley, et al. 2012. “Germline Mutations in HOXB13 and Prostate-Cancer Risk.” *The New England Journal of Medicine* 366 (2): 141–49. https://doi.org/10.1056/NEJMoa1110000.

173. Brechka, Hannah, Raj R. Bhanvadia, Calvin VanOpstall, and Donald J. Vander Griend. 2017. “HOXB13 Mutations and Binding Partners in Prostate Development and Cancer: Function, Clinical Significance, and Future Directions.” *Genes & Diseases* 4 (2): 75–87. https://doi.org/10.1016/j.gendis.2017.01.003.

174. Hamid, Syed Muhammad, Seher Cicek, Selda Karamil, Mert Burak Ozturk, Bilge Debelec-Butuner, Burcu Erbaykent-Tepedelen, Lokman Varisli, Ceren Gonen-Korkmaz, Kutsal Yorukoglu, and Kemal Sami Korkmaz. 2014. “HOXB13 Contributes to G1/S and G2/M Checkpoint Controls in Prostate.” *Molecular and*

Cellular Endocrinology 383 (1–2): 38–47. https://doi.org/10.1016/j.mce.2013.12.003.

175. Risbridger, Gail P., Ian D. Davis, Stephen N. Birrell, and Wayne D. Tilley. 2010. "Breast and Prostate Cancer: More Similar than Different." *Nature Reviews Cancer* 10 (3): 205–12. https://doi.org/10.1038/nrc2795.

176. Kach, Jacob, Suzanne D. Conzen, and Russell Z. Szmulewitz. 2015. "Targeting the Glucocorticoid Receptor in Breast and Prostate Cancers." *Science Translational Medicine* 7 (305): 305ps19-305ps19. https://doi.org/10.1126/scitranslmed.aac7531.

177. West, Diana C., Deng Pan, Eva Y. Tonsing-Carter, Kyle M. Hernandez, Charles F. Pierce, Sarah C. Styke, Kathleen R. Bowie, Tzintzuni I. Garcia, Masha Kocherginsky, and Suzanne D. Conzen. 2016. "GR and ER Co-Activation Alters the Expression of Differentiation Genes and Associates with Improved ER+ Breast Cancer Outcome." *Molecular Cancer Research : MCR* 14 (8): 707–19. https://doi.org/10.1158/1541-7786.MCR-15-0433.

178. Skor, Maxwell N., Erin L. Wonder, Masha Kocherginsky, Anju Goyal, Ben A. Hall, Yi Cai, and Suzanne D. Conzen. 2013. "Glucocorticoid Receptor Antagonism as a Novel Therapy for Triple-Negative Breast Cancer." *Clinical Cancer Research : An Official Journal of the American Association for Cancer Research* 19 (22): 10.1158/1078-0432.CCR-12–3826. https://doi.org/10.1158/1078-0432.CCR-12-3826.

179. Hickey, Theresa E., Connie M. Irvine, Heidi Dvinge, Gerard A. Tarulli, Adrienne R. Hanson, Natalie K. Ryan, Marie A. Pickering, et al. 2015. "Expression of Androgen Receptor Splice Variants in Clinical Breast Cancers." *Oncotarget* 6 (42): 44728–44. https://doi.org/10.18632/oncotarget.6296.

180. Ferguson, Donna C., Douglas A. Mata, Timothy Ky Tay, Tiffany A. Traina, Ayca Gucalp, Sarat Chandarlapaty, Timothy M. D'Alfonso, et al. 2022. "Androgen Receptor Splice Variant-7 in Breast Cancer: Clinical and Pathologic Correlations." *Modern Pathology: An Official Journal of the United States and Canadian Academy of Pathology, Inc* 35 (3): 396–402. https://doi.org/10.1038/s41379-021-00924-5.