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Novel therapeutic approaches to metastatic castration- resistant prostate cancer

Neha Yashpal Tuli

A Literature Review in the Program in Pharmacology
Submitted to the Faculty of the
Graduate School of Basic Medical Sciences
in Partial Fulfillment of the Requirements
for the Degree of Master of Science
at New York Medical College

2015

Novel therapeutic approaches to metastatic castration - resistant prostate cancer

Neha Yashpal Tuli

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Sponsor

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Reader

3/31/2015

Date of approval

Acknowledgement

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List of Abbreviations

ACTH	Adrenocorticotropic hormone
ADT	Androgen deprivation therapy
AED	Androstenedione
AR	Androgen receptor
AUC	Area under concentration-time curve
BTG	British technology group
Cmax	maximum concentration
CYP17A	Cytochrome P450 17-alpha hydroxylase
DBD	DNA binding domain
DES	Diethylstibesterol
DHEA	Dehydroepiandrosterone
DHT	Dihydrotestosterone
ECOG	Eastern Cooperative Oncology Group
FDA	Food and Drug Administration (United States)
FSH	Follicle stimulating hormone
GM-CSF	Granulocyte macrophage colony-stimulating factor
GnRH	Gonadotropin releasing hormone
HR	hazards ratio
ICR	Institute of cancer research
LBD	Ligand binding domain
LH	Luteinizing hormone
LHRH	Luteinizing hormone releasing hormone
LHRH-R	Luteinizing hormone releasing hormone receptor
mCRPC	Metastatic castration resistant prostate cancer
OS	Overall survival
PAP	Prostatic acid phosphatase
PS	Performance status
PSA	Prostate specific antigen
rPFS	radiologic progression-free survival
ULN	upper limit of normal

Abstract

Many pharmacological interventions are used for prostate cancer like LHRH agonist (leuprolide, goserelin- hindering gonadal steroid synthesis), agents directly acting on androgen receptors e.g. bicalutamide, flutamide etc. But for metastatic castration resistant prostate cancer (mCRPC), docetaxel(a taxane) was the only drug used about five years ago. Hence, other pharmacological approaches were required against castration resistant prostate cancer. Continued targeting of androgen-dependent pathway proved to be a new and efficacious approach for treating mCRPC. Two new drugs, Abiraterone and Enzalutamide(MDV3100) target these pathways.

Abiraterone is an irreversible and potent inhibitor of CYP17 (cytochrome P450 17α hydroxylase), essential enzyme for androgen biosynthesis. Its use alone might lead to increased levels of adrenocorticotropic hormone production due to positive feedback to hypothalamus-pituitary axis; hence it is usually co-administered with a glucocorticoid. Phase I/II clinical trial study shows that this therapy led to a decline in prostate specific antigen levels by ≥50% (most of the patients previously received anti androgen therapy). It also showed improved PSA levels in patients with failed prior combined androgen deprivation therapy (ADT) and up to two cytotoxic therapies. Abiraterone with prednisolone increases the survival rate in pre and post chemotherapy treated patients. On the other hand, glucocorticoid and mineralocorticoid receptor antagonists may activate mutant androgen receptor (AR), which can be inhibited by bicalutamide or enzalutamide. Hence combination therapy is important to prevent resistance of a single drug.

Enzalutamide (MDV3100) is a direct inhibitor of androgen receptors (ARs). It binds to AR irreversibly with higher affinity than bicalutamide which leads to decreased nuclear translocation and transcription of putative mitogenic signals. Phase I/II clinical trials has showed that enzalutamide is well tolerated drug, lowering PSA levels in chemotherapy naïve patients as well as patients with prior cytotoxic therapy.

Identification of biomarkers modulated by these agents is the next step to recognize patients who would respond to this therapy. Other agents that mimic these above mentioned drugs like dasatinib, BMS-641988 etc are in Phase II and Phase I trials respectively. The collective data reveal multiple promising therapies for metastatic castration resistant prostate cancer

Novel therapeutic approaches to metastatic castration - resistant prostate cancer

Introduction to Prostate Cancer

Prostate cancer is the second most frequently diagnosed cancer and the sixth leading cause of cancer death in males, accounting for 14% (903,500) of the total new cancer cases and 6% (258,400) of the total cancer deaths in males in 2008(Ferlay et al., 2010). Higher incidence rates are recorded in the developed countries of Europe, North America, Australia and New Zealand due to wide utilization of PSA testing that detects clinically relevant tumors as well as slow growing difficult to diagnose prostate tumors. The incidence rates still vary worldwide depending on the facilities for diagnosis of prostate cancer. Mortality rates vary as well reflecting genetic susceptibility in different races; with patients of African descent in the Caribbeans showing the highest prostate cancer related mortality(Figure 1)(Jemal et al., 2011). Some of the well established risk factors for prostate cancer include advancing age, race(black) and family history(Platz EA, 2006).

Knowledge of the normal anatomy and physiology of prostate gland provides insight into the development of prostate cancer and the changes that occur due to the cancer or the treatment.

The prostate is a small gland as part of male reproductive system (Figure 2)(Centers for Disease Control and Prevention, Division of Cancer Prevention and Control, 2015). It is located in the pelvis, sits under the urinary bladder and in front of the rectum. A portion of the urethra is surrounded by prostate gland. The urethra is a narrow tube which runs from the bladder through the length of penis and carries both urine and semen. Diseases of the prostate affect symptoms like urination, ejaculation, and sometimes defecation due to its location.(Aumuller, 1979)(K. Moore and Dalley, 1999).The prostate gland requires male sex hormone (androgens) for its proper development and functionality. Androgens include testosterone made in testes, dihydroepiandrosterone made in the adrenal glands as

well as dihydrotestosterone. Mutations of prostate epithelial cells due to exposures to high levels of androgens, mainly testosterone, can stimulate prostate cell proliferation leading to generation of prostate cancer. Hence it can be said that prostate cancer is androgen- dependent.

Prostate cancer possesses heterogeneity, within individuals as well as across the affected population (Mostofi et al., 1993), which can be attributed to accumulation of random genetic hits over time. However, a high incidence rate of prostate cancer suggests a single unifying factor. This age related and prostate specific factor might be a carcinogenic driving force or prevalent susceptibility aspect, but still it is responsible for generation of molecularly heterogeneous tumors (O'Hanlon Brown and Waxman, 2012a).

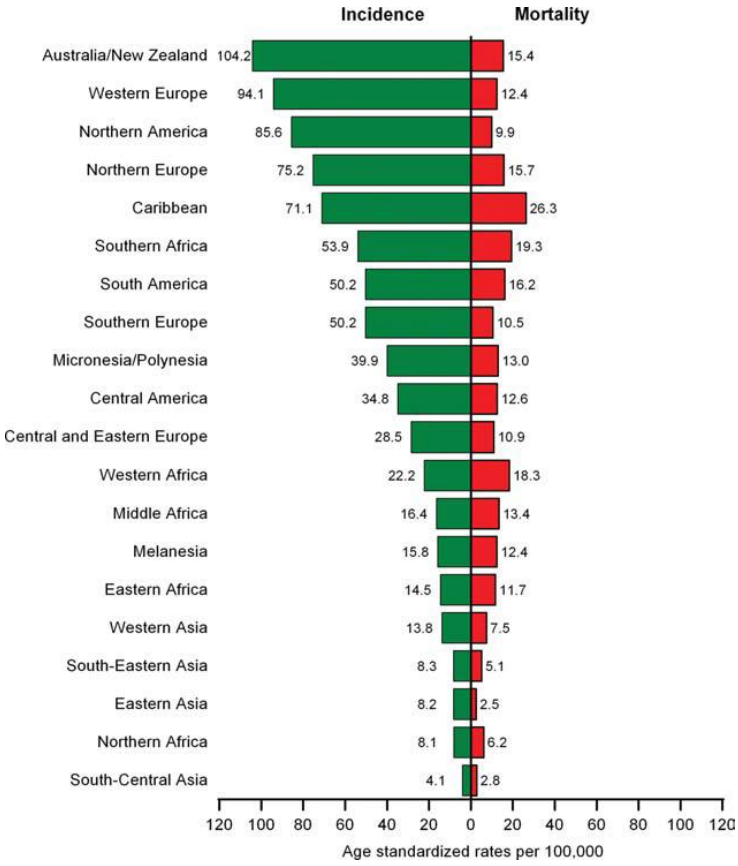


Figure 1: Age-Standardized Prostate Cancer Incidence and Mortality Rates by World Area. From(Ferlay et al., 2010)

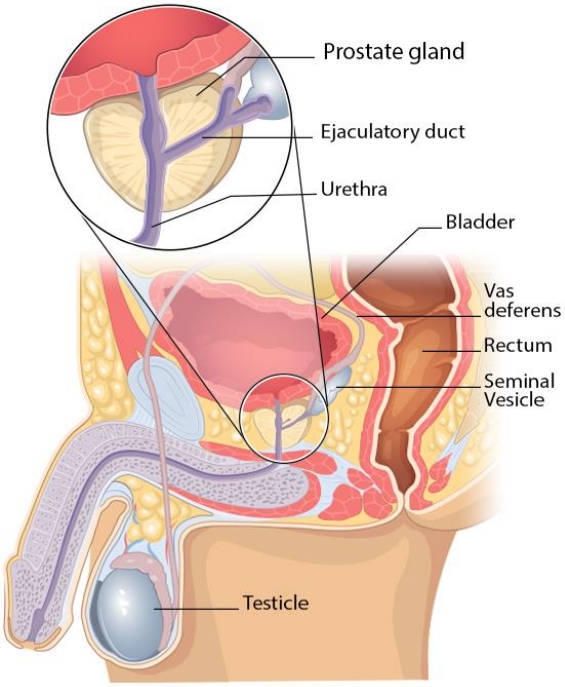


Figure 2: Anatomy and location of prostate.)(Centers for Disease Control and Prevention, Division of Cancer Prevention and Control, 2015)

Current therapies for hormone sensitive prostate cancer:

Androgen Ablation Therapy

In 1786, John Hunter became a pioneer in using androgen ablation to control prostate disease. Variation in the size of animal testicles and prostate due to castration led to the conclusion that there existed a direct connection between the testes and secondary sexual organs (Denmeade and Isaacs, 2002)(Palmer, 1837).These studies were confirmed by the surgeon W White in 1893 after observing the atrophy of glandular structures and decrease in weight as well as size of prostate gland in dogs after castration. Based on these results, he suggested castration as treatment for urinary obstruction disorders (White, 1893)(Denmeade and Isaacs, 2002).Medical castration with oral estrogens became thefirst effective systemic treatment for prostate cancer after Robert Moore and Allister Mclellan confirmed the atrophic effect of estrogen injection on prostate epithelium(R. Moore and McClellan, 1938).

Fifty years ago, the typical patient with metastatic prostate cancer was a man in hisearly seventies who was diagnosed with metastases to the bone and/or soft tissues. A significant discovery was made in the 1940s when Charles Huggins found that metastatic prostatecancer responds to androgen-ablation therapy. This brought about the new era ofprostate cancer therapy(Huggins et al, 1941). The knowledge acquired from these studies laid the foundation for androgen ablation in becoming the mainstay treatment for prostate cancer therapy (Figure 3)(Denmeade and Isaacs, 2002)

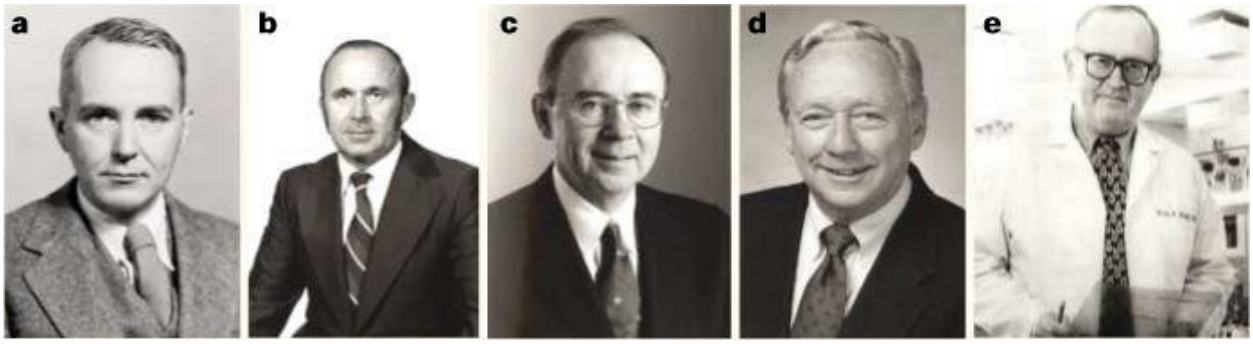


Figure 3. Prostate cancer research pioneers
a| Charles Huggins discovered that prostate cancers respond to androgen therapy. **b**| Andrew Schally determined the structure of luteinizing hormone-releasing hormone and developed the means to synthesize it. **c**| Patrick Walsh developed a modified technique for radical retropubic prostatectomy. **d**| Malcolm Bagshaw investigated the use of radiation therapy for prostate cancer. **e**| Gerald Murphy evaluated the efficacy of chemotherapy in patients with hormone-refractory prostate cancer, and his lab discovered prostate-specific antigen. From (Denmeade and Isaacs, 2002)

There exists a relationship between the hypothalamus, pituitary, testes and prostate gland. Figure 4 clearly shows the interplay of signals between these organs for production or suppression of testosterone by a feedback loop system that affects the functionality of prostate gland(Denmeade and Isaacs, 2002).

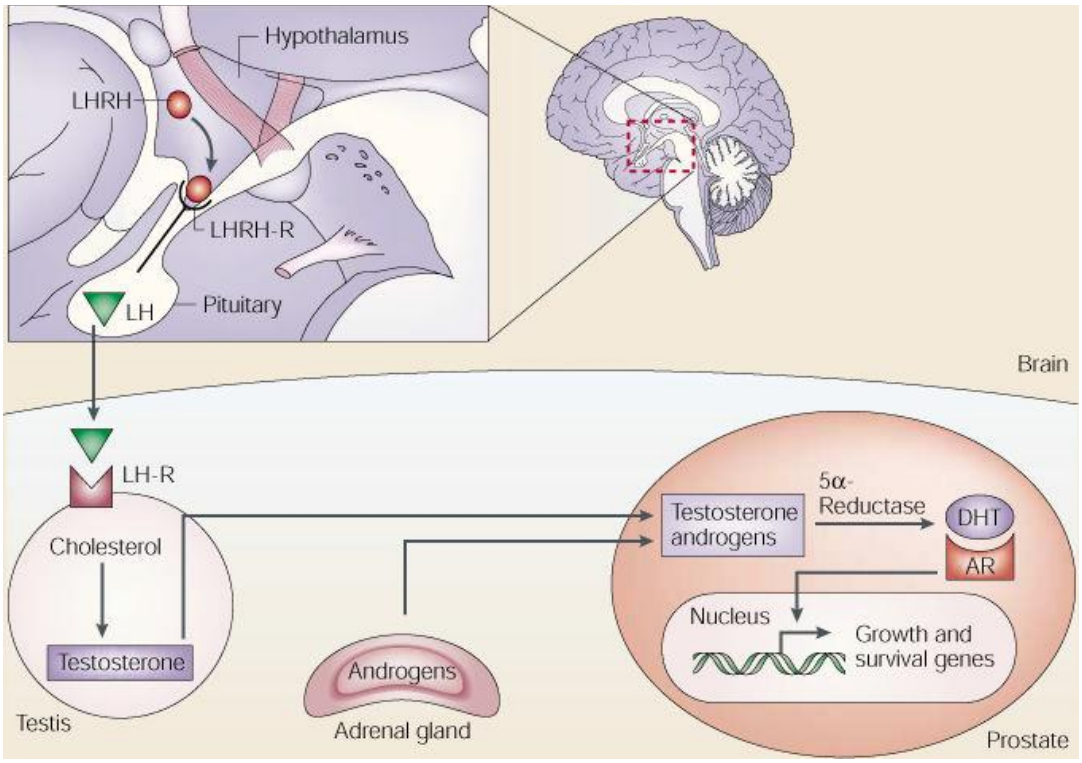


Figure 4: Androgen production and action From (Denmeade and Isaacs, 2002)
In the hypothalamus, androgens bind to the androgen receptor (AR) to stimulateproduction of luteinizing hormone -releasing hormone (LHRH), also known as gonadotropin-releasing hormone. LHRH travels tothe pituitary where it interacts with LHRH receptors (LHRH-Rs). This interaction stimulates the release of LH. LH that is released by the pituitary binds to LH receptors(LH-R) in the testes, inducing production of testosterone, which is then synthesized from cholesterol. Testosterone enters prostate cells, where it is converted to dihydrotestosterone (DHT) by the enzyme 5α reductase. DHT binds tightly to the AR and entersthe cytoplasm, and the complex translocates to the nucleus where it activates transcription of genes which regulate cell growth and survival. Increased testosterone levelscan also decrease LHRH and LH production through negative feedback loops, therebymaintaining serum testosterone at physiological levels. The adrenal gland can alsoproduce androgens.

Early stages of prostate cancer were treated by surgery and radiation. However, the treatment for advanced disease requires suppression of testosterone production. A randomized clinical study was organized by Veterans Administration Cooperative Urologic Research Group (VACURG, 1967)in 1960s to compare the effectiveness of medical castration by diethystilbesterol (DES, an oral estrogen) and orchidectomy as androgen ablation treatment for prostate cancer. The study concluded that both were equally efficacious. It also revealed that there was an increased risk of cardiovascular and thromboembolic events on treating patients with oral estrogen in attempt to decrease their serum testosterone levels. Although orchidectomy was a simple and safe procedure but

the psychological effects of the procedure made patients reject it as a treatment option(Wilson, Meethal, Bowen, and Atwood, 2007).

Medical (oral estrogen) and surgical castration led to androgen ablation which offered palliative benefit with patients experiencing less pain, increase in weight, appetite and hematocrit. However further studies revealed that androgen ablation by castration or oral estrogen did not offer a permanent cure to the disease. Infact oral estrogen therapy to diminish serum testosterone levels caused significant cardiovascular and thromboembolic toxicity (Huggins et al, 1941)(Denmeade and Isaacs, 2002). As shown in Figure 4, low levels of androgen are also produced by adrenal glands which aids in cancer progression. Adrenalectomy or hypophysectomy did offer transient palliative relief for tumor growth, but the risk and complexity of major surgery outweighed the minor reprieve patients achieved from the symptoms.

The next novel approach was manipulation of the hypothalamus-pituitary-testes axis. Andrew Schally and colleagues discovered**LHRH agonists (class of gonadotropin releasing hormone GnRH agonists)**which regulate LH and FSH. These potent GnRH analogues were initially speculated to increase fertility but instead induced paradoxical antifertility effects. Human studies in late 1970s revealed that LHRH agonists decreased male testosterone steroidogenesis. Further investigation led to use of LHRH agonists as a treatment modality in androgen dependent prostate cancer(Sandow et al., 1978). Administration of LHRH agonist produced a transient increase in serum testosterone initially, known as 'testosterone flare' which caused bone pain, cord compression, uremia, paralysis or in isolated cases death (Wilson et al., 2007)(Figure 5a(Brawer, 2001)). Prolonged administration of these exogenous agents led to suppression of endogenous gonadotropin secretion and down regulation of LHRH receptors in pituitary (Figure 5b, (Brawer, 2001)). This resulted in desensitization of gonadotrophs and repression of the circulating levels of luteinizing hormone LH and follicle stimulating hormone FSH (Sandow et al., 1978)(Vilchez-Martinez et al., 1979). Low levels of circulating LH and FSH, along with downregulation of gonadal LHRH receptors led to diminished production of testosterone to <50 ng/dl by 14-28 days (Wilson et al., 2007).

This fall in serum testosterone levels is equivalent to those caused by castration. Daily administration of LHRH agonists for treatment of advanced prostate cancer showed significant improvement in symptoms and general health of patients as observed by 75% decrease in serum testosterone levels, normalization of plasma acid-phosphatase levels, and a marked reduction in cancer associated bone pain which is equivalent to surgical castration or oral estrogen therapy(Tolis et al., 1982a).

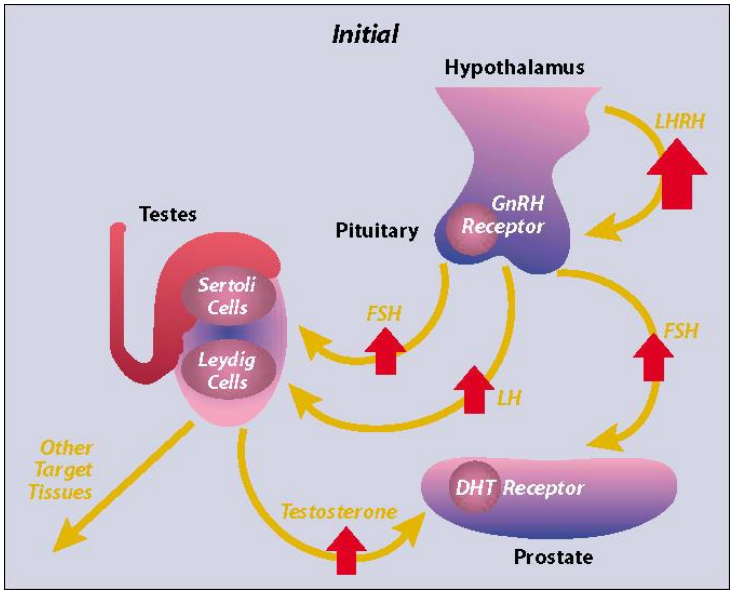


Figure5a: From (Brawer,M.K. 2001) LHRH agonist mediated initial rise in LH and FSH with a resultant rise in serum testosterone

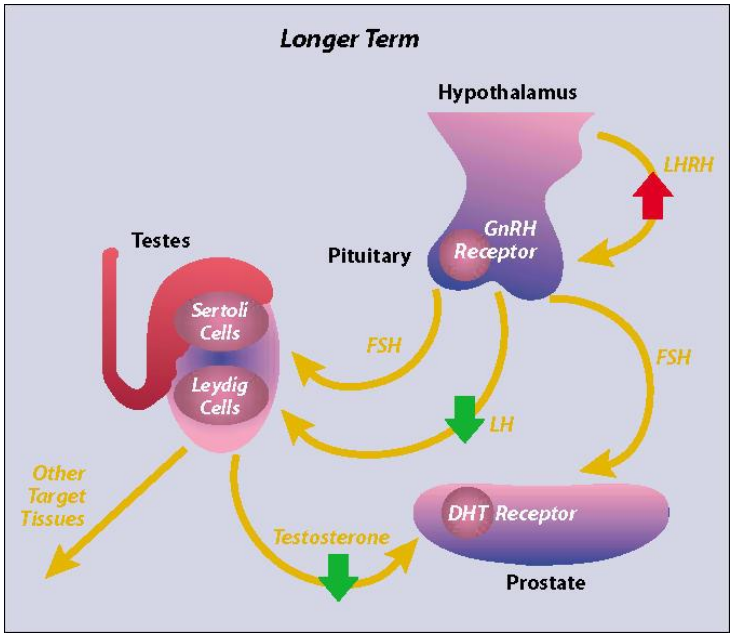


Figure 5b: From (Brawer,M.K. 2001)

Long term LHRH agonists cause downregulation of pituitary LHRH receptors with subsequent reduction in LH/FSH release. This results in castrate levels of testosterone.

These properties of LHRH agonist led to their development for clinical use. The drugs included in this category are leuprolide (Lupron),goserelin (Zoladex), buserelin, and nafarelin. Endogenous LHRH is secreted in pulsatile fashion by being under constant regulation of the negative feedback to hypothalamus pituitary axis and has a stimulatory effect on release/synthesis of LH and FSH. On the other hand, continuous exogenous synthetic LHRH agonists have an inhibitory action on testicular steroidogenesis. Side effects include hot flushes, loss of libido and impotence, but cardiovascular or thromboembolic toxicity was not observed as seen with estrogen treatment. Hence these peptides proved to be safer than previous approaches. Randomized trials designed to compare the efficacy of various hormone ablative therapies revealed that LHRH agonists were as effective as orchiectomy or estrogen administration (Denmeade and Isaacs, 2002).

The role of ligand-receptor interaction of the androgen receptor (AR) signaling axis in advanced prostate cancer validate AR as a therapeutic target throughout the clinical progression of prostate cancer. This served as the rationale to develop ADT(Androgen deprivation therapy), the first line systemic treatment for prostate cancer, which has been the standard of care for patients with non-organ confined prostate cancer for last seventy years (Miyamoto et al., 2004).Hence, amongst the androgen ablative therapies, LHRH agonists have become the preferred mode of treatment for hormone sensitive prostate cancer in many countries, including the United States.

The **second line hormone therapy** consists of antifungal agent **ketoconazole,** which inhibits sterol synthesis in fungi and in humans, along with low dose corticosteroid for patients who are unresponsive to androgen ablation and LHRH agonist (Denmeade and Isaacs, 2002). This modality of treatment was developed to suppress adrenal steroidogenesis as an alternative to adrenlectomy. However, there was complete inhibition of corticosterone synthesis as well which emphasized the need for co-administration of low dose corticosteroid along with ketoconazole (Pont et al., 1982). Aminoglutethimide, an adrenal aromatase inhibitor, is another drug with similar effects, but the use is limited due to its toxicity.

Several **LHRH antagonists**, like degarelix, cetrorelix (Cetrotide), abarelix and ganirelix offer rapid reduction in serum testosterone levelswithout producing the testosterone flare, normally associated with the early stage of LHRH agonist therapy. They were tested in clinical trials as treatment of advanced prostate cancer to directly inhibit the LHRH receptors and maintain castrate level of testosterone (Crawford and Hou, 2009)(Denmeade and Isaacs, 2002). The first LHRH antagonist, abarelix showed more rapid achievement of clinical castrate levels of testosterone compared to LHRH agonist monotherapy or combination therapy with bicalutamide. But it did possess histamine releasing properties which gave rise to potential life threatening immediate onset systemic allergic reaction (US Food and Drug Administration,). On the other hand, Degarelix, LHRH antagonist approved by US FDA in 2008 has acceptable safety profile and avoid testosterone surge. Phase III studies also state that degarelix suppresses testosterone, PSA as well as FSH levels more quickly than leuprolide, and maintains androgen deprivation for one year. Degarelix therapy did not instigate immediate or late onset systemic allergic reactions, unlike abarelix. Hence it is considered to be a potential new effective therapy for prostate cancer patients(Klotz et al., 2008).

The androgen flare occurring with initiation of GnRH agoinst therapy can be prevented by short-term administration of antiandrogens (Kuhn et al., 1989). **Non-steroidal anti-androgen** like bicalutamide, flutamide or nilutamide(Figure 6); are alternatives to surgical or medical castration for locally advanced prostate cancer. Bicalutamide (Casodex) is a competitive androgen receptor antagonist that inhibits androgen-regulated prostate cell growth and function. It is administered orally as a once-daily dose of 150mg. But such a monotherapy did not offer much benefit in metastatic disease with respect to overall survival. Bicalutamide therapy was equivalent to the effects of castration, but it was better tolerated and offered higher health-related quality of life scores for sexual interest and physical capacity compared with surgical or medical castration(Wellington and Keam, 2007).

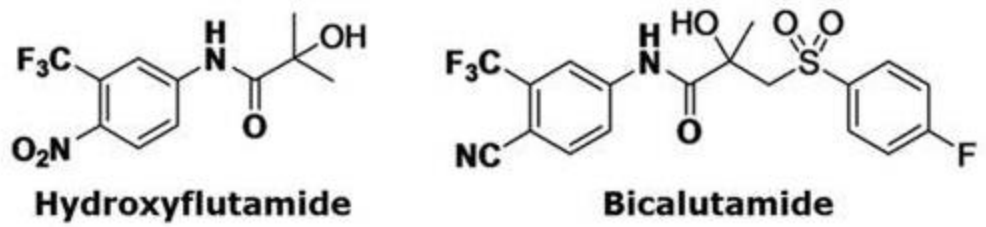


Figure 6: From (Song et al., 2012) Structure of bicalutamide and hydroxyl flutamide

However, **combinational androgen ablation** using GnRH analogus (LHRH agonist-leuprolide) with non- steroidal anti androgens block synthesis and binding of gonadal and non-gonadal androgen to their receptor, preventing androgen signaling to prostate tumor. This leads to maximal androgen blockade(Schmitt et al., 2001)(Dupont et al., 1993)(Wilson et al., 2007).Majority of previous small clinical trials conducted over period of time did not report any significant increase in 5 year survival with this approach. However, placebo controlled double blinded study conducted by National Cancer Institute suggest a five percent improvement in five year survival rate of men with prostate cancer. There was also an improvement in progression- free survival at one year(Crawford et al., 1989). Other investigations also demonstrated a reduction in prostate specific antigen levels with combination therapy versus monotherapy. The combinational androgen ablation was over all very well tolerated with improvement in quality of life, except for diarrhea (especially with flutamide),(Schellhammer et al., 1995).

Intermittent hormonal therapy has been postulated as another approach to allow tumors to recover their ability to undergo apoptosis after they become androgen independent with GnRH agonist treatment. This approach consists of about 8months of initial androgen deprivation (time required for maximal loss of tumor mass) followed by removal of GnRH agonist treatment. This allows the testosterone levels to return back to pre-castration levels, with androgen independent cells losing their ability to grow. This approach has been tested in animals, showing a threefold prolongation of progression to hormone refractory state (Tolis et al., 1982b)(Heidenreich et al., 2008).

Cytotoxic chemotherapy for prostate cancer includes use of **taxanes.** Microtubules are highly dynamic cytoskeletal fibres composed of tubulin subunits and extremely important in mitotic cell division and interphase cellular functions such as intracellular transport and signaling. Slow growing solid tumors such as prostate cancers have low mitotic index with low levels of cellular death on cytotoxic chemotherapy treatment. Hence in general such therapies offer little clinical benefit with major toxicities. Taxanes induce cytoskeletal microtubule stabilization by binding to β-tubulin which in turn leads to mitotic arrest and apoptotic cell death in actively dividing cells (Jordan and Wilson, 2004). Recent studies suggest that taxanes can also inhibit AR signaling via stabilization of its microtubules for AR trafficking(Darshan et al., 2011). The antimitotic effect of taxanes cannot be the sole contributor to its clinical activity, hence it became important to understand the effect of taxanes on microtubular interphase. It was observed that taxanes regulate the interphase microtubule functions along with AR signaling and trafficking pathways. Taxanes inhibit ligand-induced AR nuclear translocation and downstream transcriptional activation of AR target genes such as prostate-specific antigen.(Mostaghel, 2014a)(Darshan et al., 2011).**Docetaxel** is a taxane that stabilise microtubules and prevent tubulin depolymerisation leading to G2/M arrest and apoptosis. In TAX327(a phase III randomized clinical trial, 2004) docetaxel 75mg/m2 plus prednisolone significantly improved the overall survival from 16.5 to 18.9 months (p=0.009) (Tannock et al., 2004)(O'Hanlon Brown and Waxman, 2012a). This was accompanied by decrease in pain experienced by the patients (pain response rate from 22% to 35%, p=0.01), and improvement in quality of life(Tannock et al., 2004)(Petrylak et al., 2004).**Cabazitaxel** is another taxane with similar mechanism of action. It has shown promising activity in pre-clinical studies, being capable of producing a complete response in DU145 xenograft tumors. In 2004, the TAX327 and SWOG9916 studies showed a survival advantage of docetaxel over mitoxantrone in men with metastatic castration resistant prostate cancer. In 2010, the TROPIC study demonstrated a survival advantage of cabazitaxel over mitoxantrone in men previously treated with docetaxel(J. S. de Bono et al., 2010). Thus taxanes being the only class of cytotoxic chemotherapy for prostate cancer that inhibit the AR signaling pathway via stabilization of microtubules,

has made them as effective as other drugs affecting the AR axis in reaching castration state.

Radium-223 is a radiopharmaceutical that has demonstrated improved overall survival relative to placebo, but not improved time to PSA or radiographic progression(Parker et al., 2013).

The first validation of active immunotherapy as a viable approach to cancer treatment was approval of **Sipuleucel T** by FDA in 2010 for advanced prostate cancer also known as **Provenge**(Graff and Chamberlain, 2014). Sipuleucel-T is designed to induce a systemic immune response against the patient's prostate cancer cells, which express PAP(Prostatic acid phosphatase – phosphatase enzyme specific to prostate tissue)(Goldstein, 2002). GM-CSF is the other crucial component which upregulates key immune functions molecules like cytokines and costimulatory molecules(Jubinsky et al., 1994).The PA2024 (PAP with GM-CSF form recombinant fusion protein)-loaded antigen presenting cells (APCs) make up the active component of sipuleucel-T(Patel PHet al., 2008).T-cells bind the processed recombinant antigen on the surface of the APC. Once bound, the T-cell activates circulating T-cell-mediated destruction of tumor cells by immunogenic cell death(Graff and Chamberlain, 2014)(Hammerstrom et al., 2011)(Figure 7 from(Garcia and Dreicer, 2011)

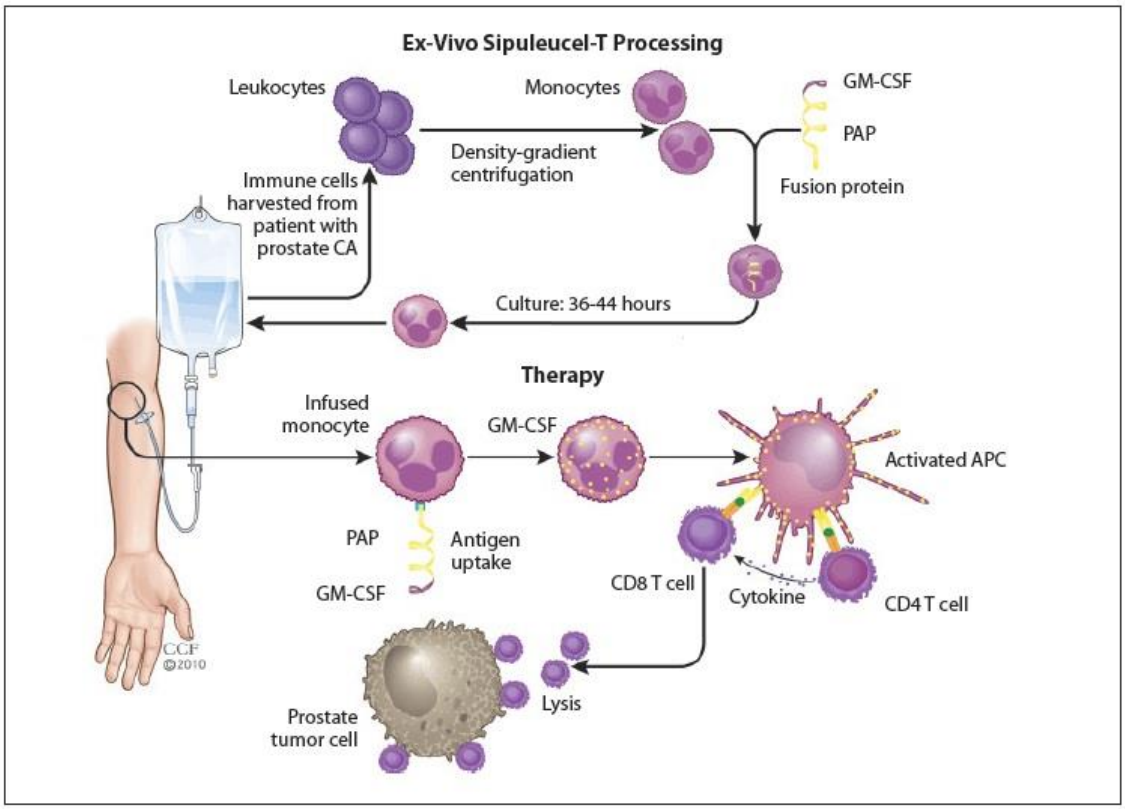


Figure 7: From(Garcia and Dreicer, 2011): The two steps involved in sipuleucel-T therapy:(1) harvesting the patient's dendritic cells and then pulsing these ex vivo with a recombinant fusion protein made of prostatic acid phosphatase (PAP) and granulocyte-macrophage colony-stimulating factor (GM-CSF); and (2) infusing the cultured cells into the patient, where the PAP-GM-CSF loaded antigen-presenting cells (APC) induce the proliferation of T-cells that recognize and target prostate tumor cells.

All four randomized clinical trials of sipuleucel-T versus control showed that sipuleucel-T was well tolerated. The adverse events that occurred more frequently in patients on sipuleucel-T are those that involved cytokine release. The IMPACT study, where only patients with asymptomatic or minimally symptomatic prostate cancer were enrolled showed significant improvement in overall survival along with two other clinical trials in mCRPC patients. Sipuleucel-T have failed to show improvement in disease-specific realms, namely disease response (PSA or radiographic) and time to progression. (Kantoff et al., 2010)[(Beer et al., 2013)(GuhaThakurta et al., 2015).

Metastatic Castration Resistant Prostate Cancer

In prostate cancer, increased androgen levels in tumor cells promote AR signaling, which regulates expression of genes encoding normal prostate functions like cell growth, survival; as well as genes with oncogenic potential(Ammannagar and George, 2015).The first-line hormonal therapy for prostate cancer targets the androgen/androgen receptor axis. Castrate testosterone levels and inhibition tumor growth is induced via surgical or medical castration using LHRH agonists and/or by targeting the ligand binding domain of AR(anti androgens).More than 90% patients respond to this first line hormonal therapy resulting in improvement in the overall survival of the patients. However patients frequently develop resistance to castration within 12-18 months on average and the tumor cells gain characteristic phenotype which leads to prostate cancer progression despite of castrate testosterone levels (levels ≤50 ng/dl). Such progressive prostate cancer coupled with metastatic spread renders the disease lethal, which is henceforth known as **metastatic castration resistant prostate cancer**–**mCRPC**(Scher and Sawyers, 2005).

The androgen receptor is a ligand-dependent transcription factor located on the X chromosome. Figure 8 shows the structure of androgen receptor, the major component of androgen signaling. The receptor is composed of an amino terminal activating domain, a hinge region, a DNA binding domain and a carboxy-terminal ligand binding domain. The inactive form of the androgen receptor (Figure 8a) resides within the cytoplasm and remains bound to heat shock proteins that prevent androgen receptor activation. The binding of androgens to the receptor leads to dissociation of the heat shock proteins and receptor phosphorylation which, in turn, leads to nuclear translocation, allowing for transcription of androgen-dependent genes(Rehman and Rosenberg, 2012).

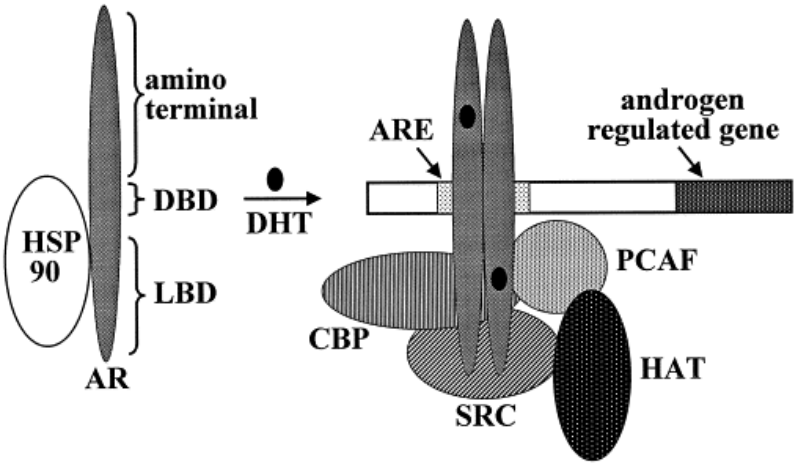


Figure 8: Outline of androgen receptor (AR) structure and function. **(a**)The unliganded AR with locations of the DNA-binding domain (DBD) and ligand-binding domain (LBD) is shown bound to the heat shock protein 90 (HSP 90) complex. Dihydrotestosterone (DHT) binding induces homodimer formation**(b)**, binding to specific DNA sequences (androgen responsive elements [ARE]), and recruitment of multiple coactivators. These include the steroid receptor coactivator (SRC) family, cyclic adenosine monophosphate response element binding protein (CREB) binding protein (CBP), p300/CBP-associated factor (PCAF), and histone acetyltransferases (HAT). From (Balk,S.P. 2002)

Figure 9 shows the various mechanisms that might be responsible for development of CRPC (Debes and Tindall, 2004a; Debes and Tindall, 2004a).Some of the proposed mechanisms include AR genomic aberrations like overexpression of the AR, AR mutations, AR activation by cross-signaling from alternative pathways, ligand-independent AR activation; altered steroidogenic enzymatic pathways leading to increased local androgen production, altered AR co-activator/co repressor interactions and/or increased expression of AR mRNA(O'Hanlon Brown and Waxman, 2012b)(Aggarwal and Ryan, 2011). The steady increase in PSA levels as well as expression of AR in CRPC proves the persistence of active AR signaling in progression of disease. It also suggests that AR is still sensitive and responsive to manipulation of androgen signaling. This means that AR can be activated in AR positive CRPC tumor cells at low/ absent ligand levels with broad AR ligand specificity. The local residual intratumoral androgen is the leading factor for mCRPC. It is produced via uptake and conversion of adrenal androgens as well as the de novo synthesis at tumor site from cholesterol precursors(Debes and Tindall, 2004a).

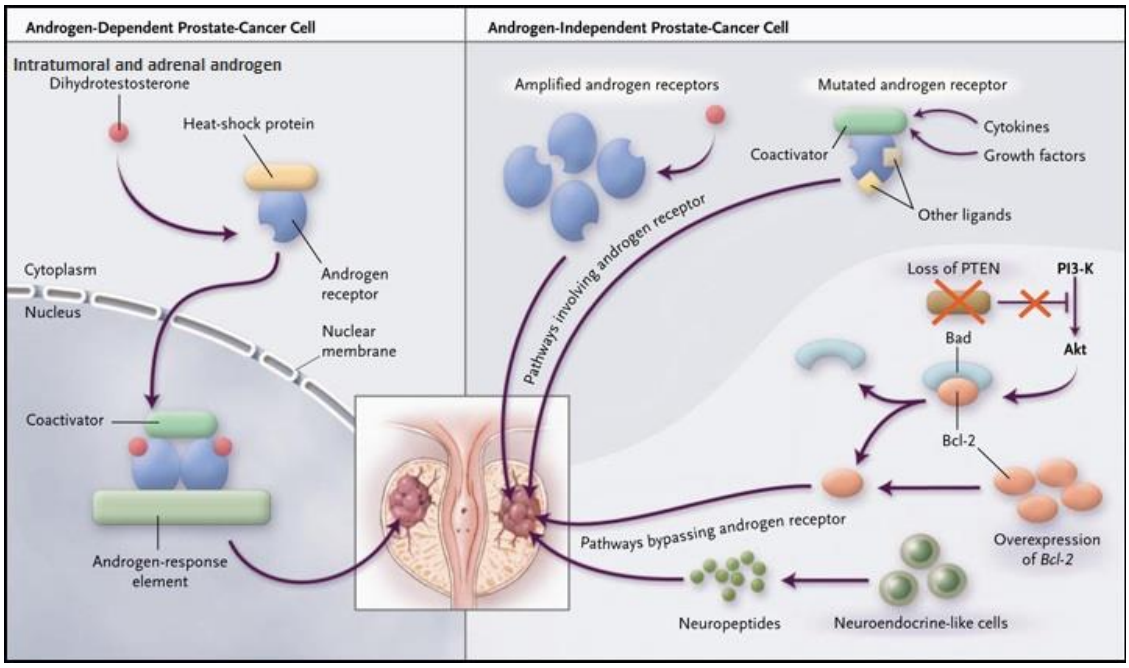


Figure 9. Mechanisms of castrate resistant prostate cancer:Adrenal androgen as well as the denovo synthesis of androgens within the tumor is responsible for generation of CRPC. The various mechanisms pertaining to androgen receptor genomic aberrations are depicted in the figure. Adapted from (Debes and Tindall, 2004a)

For men with CRPC, additional treatment is needed to help control the growth of the cancer. First generation anti-androgens were efficacious for short duration of time, possessing less affinity for androgen receptor and with greater side effect potential. Hence, there arose a need and rationale for search of novel therapies targeting molecular pathways involved in oncogenesis and tumor progression. This led to development of second generation anti-androgen therapies which include two major groups: Androgen biosynthesis inhibitors and androgen receptor blockers (Figure 10)(Ammannagar and George, 2015). Two drugs, one drug from each group, are focused in this review: namely Abiraterone and Enzalutamide.

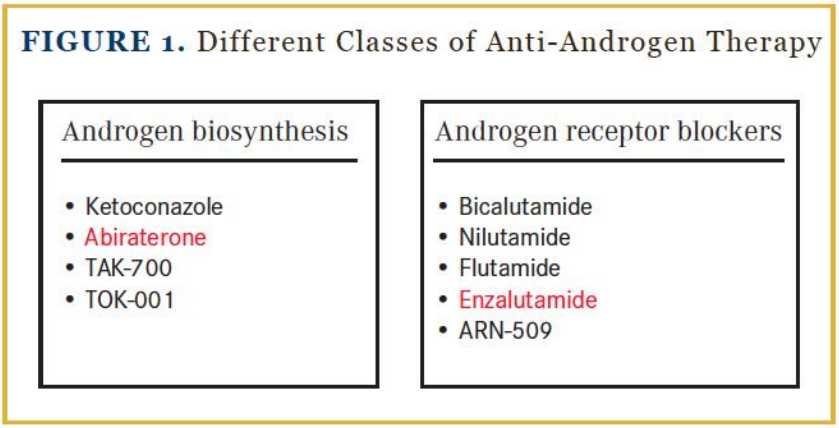


Figure 10: Different Classes of Anti-Androgen Therapy.From {{119 Ammannagar, N. 2015}})

A. **Androgen Biosynthesis Inhibitors: Abiraterone (Zytiga)**

Intratumoral cholesterol or progesterone act as precursor for de novo synthesis of androgen within the tumor or adrenal androgens are converted to intratumoral androgens. De novo synthesis of androgens require CYP17A enzyme. This enzyme is located within the endoplasmic reticulum of testis and adrenal gland. CYP17A drives the synthesis glucocorticoid (cortisol) and male sex hormones (dehydroepiandrosterone-DHEA, androstenedione-AED) via its 17 hydroxylase as well as C17,20 lyase activity(Mostaghel et al., 2014). CYP17A has become a primary target for treatment of prostate cancer since its inhibition blocks the synthesis of glucocorticoid and androgens/testosterone (Rehman and Rosenberg, 2012).Ketoconazole an antifungal agent that is also a weak inhibitor of cytochrome P450 11β-hydroxylase and CYP17Ahas been utilized for suppression of residual adrenal androgens in men with CRPC. However its limited efficacy and treatment-related side effects like hepatotoxicity, gastrointestinal toxicity and adrenal insufficiency, prompted the development of more potent CYP17A inhibitors(Mostaghel, 2014b).

Structure:

Synthesis of potent steroidal CYP17A1 inhibitors require modification of the steroid scaffold by attaching a heterocycle as a functional group onto the 17-position, which forms a strong complex with the heme iron of the enzyme. Abiraterone possesses certain structural features like a 17-(3 pyridyl) substituent together with a16,17-doublebond, which are a stringent requirement for potent inhibition(Figure 11)(Garrido et al., 2014). Certain variations in the above mentioned compound, like reductionofthe16,17-double bond diminished potency and poor inhibitory activity was noted with substituentshavinga2-or4-pyridylfunctioninstead of the commonlyused3-pyridylgroup(Garrido et al., 2014)(Potter et al., 1995). ThecrystalstructureoftheCYP17A1 complex with Abiraterone at2.6 ˚A resolution demonstrated that the C- 17-(3-pyridyl) group of the inhibitor binds to the heme iron ,and forms a 60◦ angle with the steroid

nucleus above the heme plane and packs against the central helix of CYP17A enzyme(DeVore and Scott, 2012).

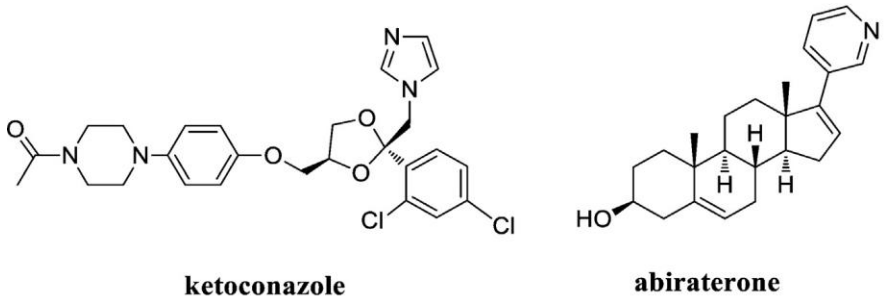


Figure 11: Structure of Abiraterone and Ketoconazole. From (Garrido et al., 2014)

Mechanism of Action:

Abiraterone acetate is an orally administered drug developed by Coughar Biotechnology and discovered by the UK institute for Cancer Research. Abiraterone is administered as a pro-drug abiraterone acetate, which has very good oral bioavailability and gets completely converted to abiraterone in the blood(Mostaghel, 2014a).Abiraterone acetate is a small molecule derived from the structure of pregnenolone which irreversibly inhibits the hydroxylase and lyase activity of CYP17A(Figure 12). Hence abiraterone inhibits both, the testosterone production in the testes and other testosterone producing tissues such as adrenal gland and tumor cells. Its potency in doing so is significantly higher than that of ketoconazole (Peer et al., 2014). Abiraterone inhibits synthesis of cortisol as well as adrenal androgen via blocking action of adrenal CYP17A, this is associated with rise of ACTH production which leads to excess production of corticosterone/ mineralocorticoid. This results in side effects due to incomplete inhibition of 17 alpha hydroxylase. On the other hand, inhibition of 17,20 lyase diverts the pathway towards androgen synthesis. Co-administration of abiraterone with low dose prednisolone/corticosterone/dexamethasone can prevent secondary rise in ACTH production which cause fluid retention, hypokalemia and hypertension as well as suppress androgens by preventing rise of androgen synthesis substrates (Attard et al., 2012)(Rehman and Rosenberg, 2012). Abiraterone is plasma protein bound, majorly albumin, and excreted in urine.

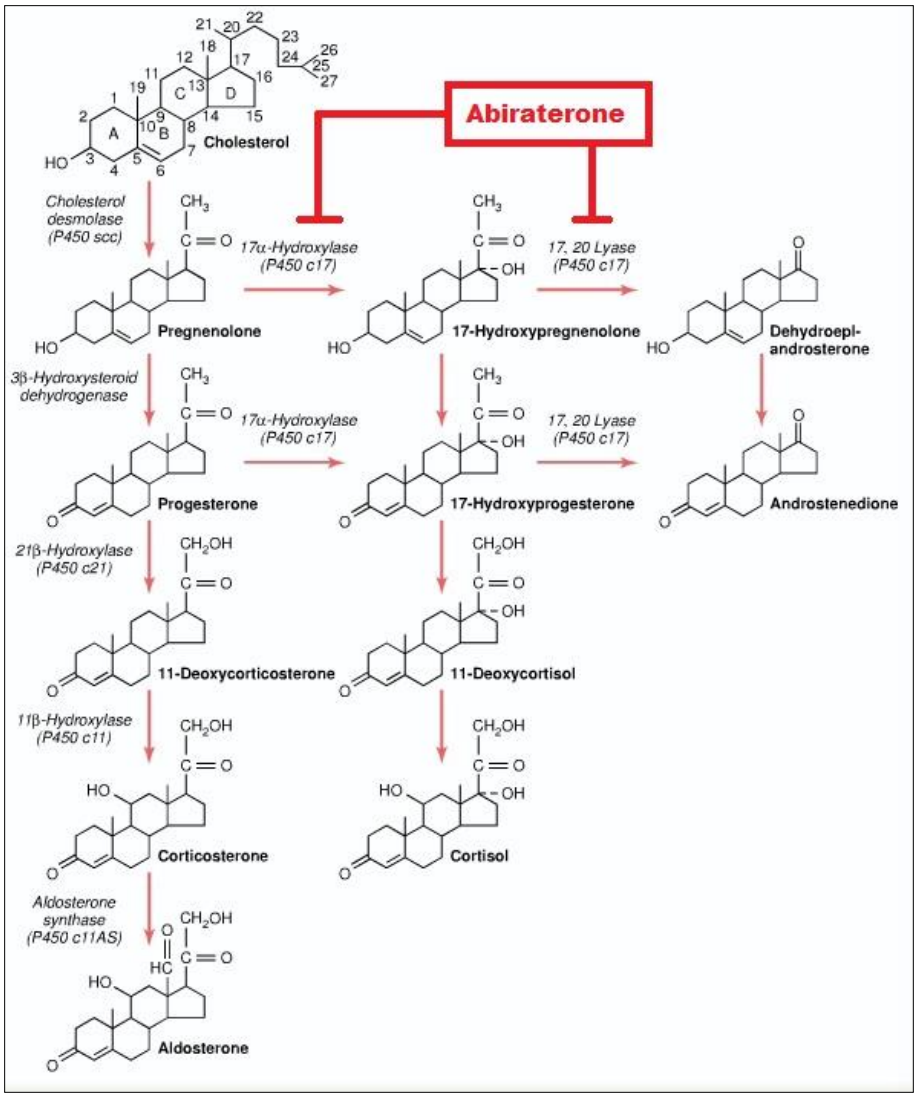


Figure 12: Schematic diagram of steroidal synthesis. Role of CYP17 enzyme and inhibition by Abiraterone. All the enzymes are in *italics.* Adapted from Guyton and Hall: Textbook of Medical Physiology

History, Development and Drug Interactions:

In 1990's scientists were searching for ways to block production of male sex hormone. They wanted to design effective and potent inhibitors of CYP17 enzyme, unlike ketoconazole which was less potent and more toxic. In 1994, Professor Mike Jarman and his colleagues, of the Cancer Research UK Cancer Therapeutics Unit at The Institute of Cancer Research (ICR) found the chemical called CB7360, later known as abiraterone. The ICR filed for patent and assigned rights for the development of abiraterone to British Technology Group, an international specialist healthcare company. In 2004, BTG

licensed abiraterone to Ortho Biotech Oncology Research and Development, a unit of Cougar Biotechnology Inc., granting worldwide exclusive rights to develop and commercialize abiraterone. In doing so the ICR gained the financial backing it needed to run the clinical trials required to prove the drug's efficacy and safety. Fourteen years after the discovery of the drug, the first phase I clinical study of abiraterone in CRPC patients, confirmed the effect of drug in causing significant tumor shrinkage and fall in PSA levels. Another phase I/II study confirmed the result from previous studies. Following this, the giant US pharmaceutical company Johnson & Johnson agreed to buy Cougar for just under £600million, gaining access to the drug as it progressed through phase III evaluation. In 2012, drug was made available on the National Health Service in England, Wales and Northern Ireland. US food and Drug Administration also approved the use of drug in the US(The Institute of Cancer Research, 2015).

Abiraterone inhibition of CYP17 enzyme which has led to its use in CRPC. But it is also a strong inhibitor of several microsomal drug metabolizing enzymes, including CYP1A2 and CYP2D6. Co-administration of abiraterone acetate with prednisolone, augmented systemic exposure of dextromethorphan (metabolized by CYP2D6) suggesting a need of caution during use of other known CYPD26 substrates (eg beta blockers, serotonin reuptake inhibitors, anti-arrythmics, neuroleptic, tramadol, tolterodine etc)(Mostaghel et al., 2014). Combinational therapies for mCRPC with abiraterone are discussed in detail in a later section.

Due to abiraterone's steroidal structure, it has some unintended activity against other AR pathways including AR itself as well as 3β hydroxysteroid dehydrogenase type I pathway, a key enzyme required for androgen synthesis. Abiraterone has been shown to inhibit two major reactions via inhibition of 3β hydroxysteroid dehydrogenase – dehydroepiandrosterone (DHEA) to androstenedione(AED) as well as 5 alpha androstanediol to testosterone along with suppression of AR regulated gene expression. Thus at the clinical dose level of 1000mg, it causes maximum inhibition of CYP17A, but it has the ability to inhibit multiple AR pathways (Mostaghel, 2014a).

Clinical Trials:

Phase I and II trials:

Phase I and II studies showed that abiraterone significantly decrease serum androgen levels as well as PSA levels denoting clinical response in chemotherapy naïve and docetaxel treated mCRPC patients. These effects were observed at all doses and without any dose limiting toxicities. Based on these trials, 1000mg was considered as the optimum treatment dose for future studies(Attard et al., 2008).

One study evaluated a small group of 21 CRPC patients, that were resistant to multiple hormonal therapies. They were treated once-daily in 28days cycles with abiraterone acetate, which escalated through five doses (250 to 2,000 mg) in three-patient cohorts. Although antitumoractivity was observed at all doses; 1,000 mg was selected as the dose for cohort expansion (n = 9), due to the plateau achieved by it in the pharmacodynamic effect.

Pharmacokinetic analysis was done on the plasma collected from these 21CRPC patients. A dose dependent increase was noted in the area under the concentration- time curve (AUC) and the maximum concentration (Cmax) but it was not proportionate($r2$=0.186and 0.049, respectively; Figs 13A and 13B). Great variations in drug absorption were observed as denoted by variations in AUC and Cmax with it reaching upto nine-folds in 1000mg cohort. The mean apparent clearance value ranged from 494.3 to 1347.2 L/hr with terminal half life consistent at mean of 10.3 hours (Fig 13C). The drug exposure was significantly increased when administered with high fat food (by 4.4-fold) compared with fasting administration (P =.049; Fig 10D).There was no significant increase in Cmax, but absorption was significantly extended after food (Attard et al., 2008).

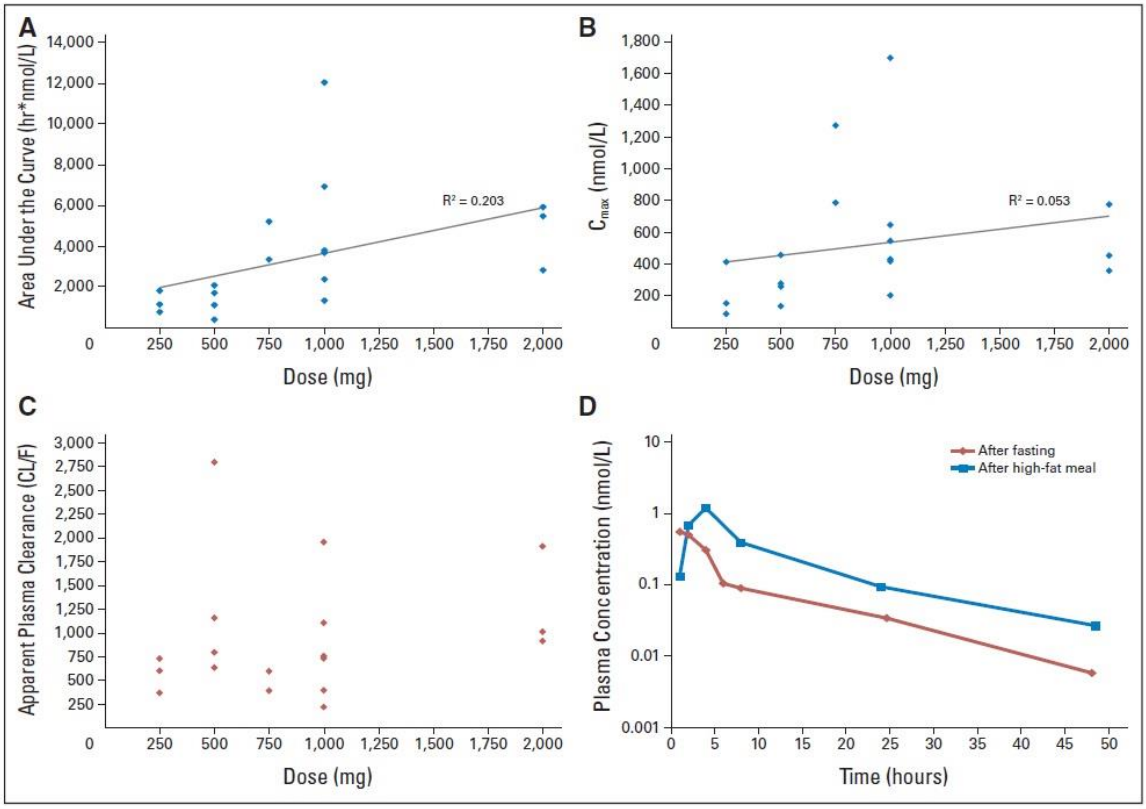


Figure 13: Pharmacokinetics of abiraterone acetate. (A) Area under the concentration-time curve versus dose in fasted patients; (B) maximum concentration (Cmax) versus dose in fasted patients; (C) apparent plasma clearance (CL/F) in fasted patients at all doses; (D) plasma concentration versus time profile in a patient treated with abiraterone acetate 1,000 mg who fasted and received abiraterone acetate after a high-fat meal. From(Attard et al., 2008)

Declines in prostate-specific antigen (PSA) of ≥30%, ≥50%, and ≥90% were observed in 14 (66%), 12 (57%), and six (29%) patients, respectively. In eugonadal men it was observed that abiraterone suppressed testosterone levels by 50%transiently, with a corresponding increase in LH levels which surpasses the inhibition of gonadal androgen synthesis. On the other hand, in castrate men, abiraterone suppressed the serum testosterone level by more than 75% (Mostaghel, 2014b). Overall, CYP17A blockade caused a decrease in testosterone, estradiol, androgenic steroids, which are regulated by adrenocorticotropic hormone (ACTH) and are upstream of 17αhydroxylase in the steroid synthesis pathway, where as an increase in ACTH was observed (Figure 14). Eplerenone (a mineralocorticoid receptor antagonist) was used to counteract the excess ACTH induced adverse effects like hypertension, hypokalemia and edema. Addition of

dexamethasone also helped in overcoming the resistance to abiraterone by inhibiting ACTH. 26% patients became resensitised to abiraterone as seen by the return of PSA response(Rehman and Rosenberg, 2012)(Attard et al., 2008).

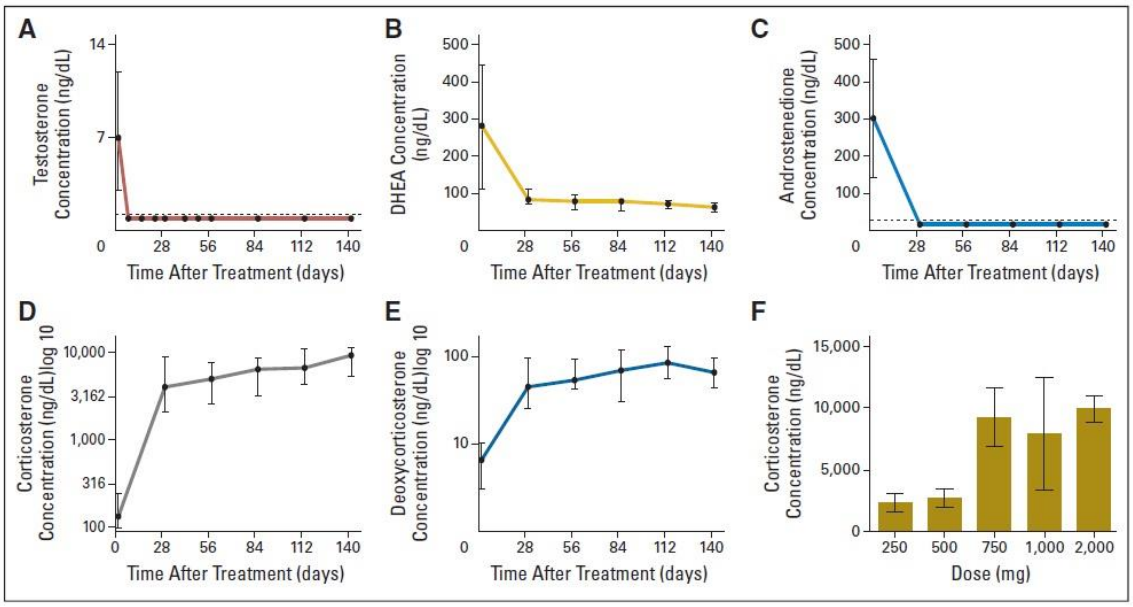


Figure 14: Pharmacodynamic end points. Treatment with abiraterone acetate results in significant suppression of testosterone, dehydroepiandrostenedione (DHEA), andandrostenedione. Median levels (error bars represent interquartile ranges) for serum levels of (A) testosterone, (B) androstenedione, and (C) DHEA at baseline and for the first 142 days of treatment. Abiraterone was found to cross-react with the DHEA assay used, which may explain the detectable levels of DHEA on abirateroneacetate. At every time point on treatment, levels of testosterone and androstenedione in all patients are less than the lower limit of sensitivity of the assay used. Median levels (log10 values on y-axis; error bars represent interquartile ranges) for serum levels of (D) corticosterone and (E) deoxycorticosterone at baseline and for the first142 days of treatment. Mean values (error bars represent 1SD) of (F) corticosterone at day 28 for every dose level (three patients who received 250 mg; two patients,500 mg; three patients, 750 mg; six patients, 1,000 mg; and three patients, 2,000 mg). From(Attard et al., 2008)

Another Phase I study of 33 chemotherapy naïve CRPC patients demonstrated that abiraterone can overcome ketoconazole resistance. It also confirmed the 1000mg daily dose of abirateone acetate for future studies. Abiraterone treatment resulted in decrease of circulating androgens, increase in deoxycorticosterone (mineralocorticoid) upstream of CYP17A and a decrease in cortisol levels. (figure 15)(Ryan et al., 2010)(Rehman and Rosenberg, 2012). In this phase I trial, similar response rates were noted in patients treated with prior ketoconazole. A decline in PSA level was observed in 47% and 64% of

patients with or without prior ketoconazole treatments respectively (Ryan et al., 2010)[(Stein, Patel, Bershadskiy, Sokoloff, and Singer, 2014)

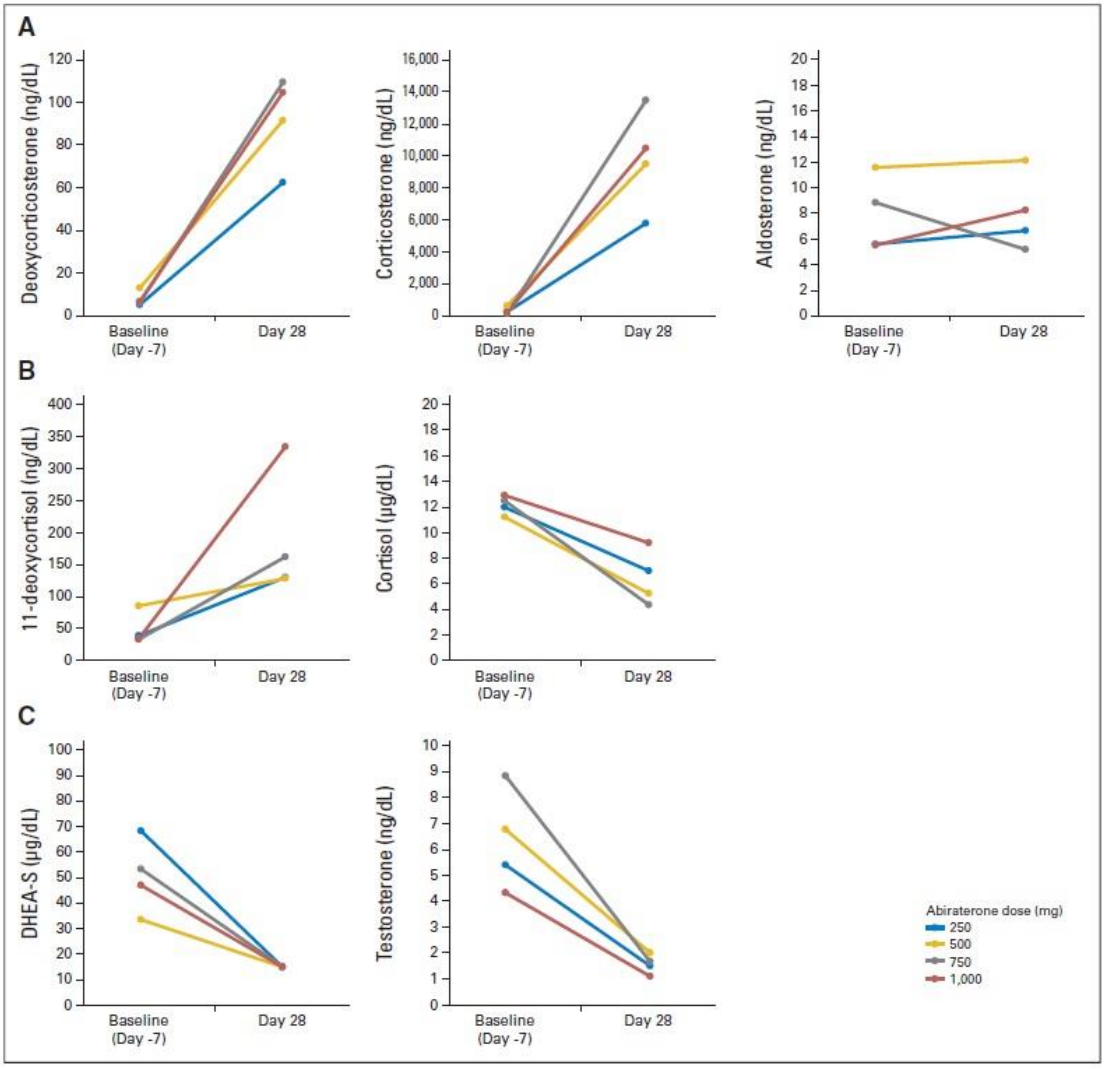


Figure 15: From(Ryan et al., 2010)Changes in mean levels of endocrine steroids from baseline to day 28 of therapy, by dose (A-C), in men with castration-resistant prostate cancer receivingabiraterone acetate. DHEA-S, dehydroepiandrosterone sulfate

In Phase II trials, 1000mg per day of abiraterone therapy was given to chemotherapy naïve patients as well as those with prior chemotherapy. These studies were conducted in UK and US (Stein et al., 2014).They looked at Prostate-specific antigen (PSA) response rate (>50% PSA decline in pretreatment PSA following chemotherapy) as a prognosis marker since it is associated with significant survival advantage in castration-resistant

prostate cancer (CRPC). One study demonstrated 67% patients with decrease in PSA levels and 32 weeks as median time to progression in chemotherapy naïve patients. Dexamethasone 0.5mg per day was added at progression of disease (Attard et al., 2009). Another study showed decrease of PSA levels in 79% patients with 71weeks of median time to progression. Prednisolone 5mg twice per day was given to all patients (Stein et al., 2014)(Ryan et al., 2011). Post chemotherapy abiraterone treatment resulted in 51% and 36% of PSA response in patients of these two trials respectively (Reid et al., 2010)(Danila et al., 2010). Prior ketoconazole treatment was received by 17% of 42 patients in the first trial, and 47% of 58 patients in the second trial. This difference might be responsible for the variations observed in the response rate between patients on the above mentioned phase II trials(Stein et al., 2014). Fatigue was the most common side effect in patients on prednisolone twice daily. The symptoms of hyperaldosteronism were relatively rare in these patients when compared to patients treated with eplerenone without prednisolone(Ryan et al., 2011). Phase II trials focused on bone scan imaging as another reliable surrogate marker of response to treatment other than PSA as indicator of treatment efficacy. Abiraterone can potentially modulate PSA levels, rendering it as less useful marker. However the utility of bone scan became questionable due to transient bone scan flare indicating false disease progression. Bone scan flare was defined as bone imaging indicating progression of disease after 3 months of therapy in presence of a ≥50% decline in PSA, with scan showing improvement three months later. Hence, this study mentioned the importance of repeat confirmatory bone scans to prevent premature discontinuation of therapy (Ryan et al., 2011).

Phase III trials:

Phase I and II trials paved a path to Phase III studies in chemotherapy-naïve (COU-AA-302) and post-docetaxel-treated men (COU-AA-301). Both trials were randomized phase III trials in patients of mCRPC (metastatic castration resistant prostate cancer) that were treated with either abiraterone plus prednisone or placebo plus prednisone.

COU-AA-301

In post docetaxel patients with mCRPC, 1195 men were randomized in a 2:1 ratio to abiraterone/prednisone (n=797) or placebo/prednisone (n=398) with a primary endpoint of OS (overall survival). The median PSA was ~130 ng/dL, 90% of patients had an Eastern Cooperative Oncology Group performance status (PS) of zero to one (figure 17), the median age was 70 years and 28% were ≥ 75years. Bone, lymph node, and visceral metastases were present in approximately 90%, 40%, and 10% of patients, respectively, and 30% of patients had received more than one prior chemotherapy regimen. Treatment was continued until clinical or radiographic evidence of progression.

The first interim analysis was conducted at a median follow-up of 12.8 months which demonstrated an OS benefit for men receiving abiraterone (14.8 months versus 10.9 months for placebo; hazard ratio [HR] 0.646; *P*,0.0001), representing a 35% reduction in risk of death and prompting the independent data monitoring committee to recommend that the study be unblinded and men on the placebo arm be offered abiraterone (J. de Bono, Logothetis, and Fizazi, 2010).It was observed after an updated analysis conducted at a median survival of 20.2 months that a median OS for abiraterone was 15.8 months versus 11.2 months for prednisone (HR 0.74; *P*<0.0001), extending the OS benefit to 4.6 months (figure 16)(Fizazi et al., 2012).

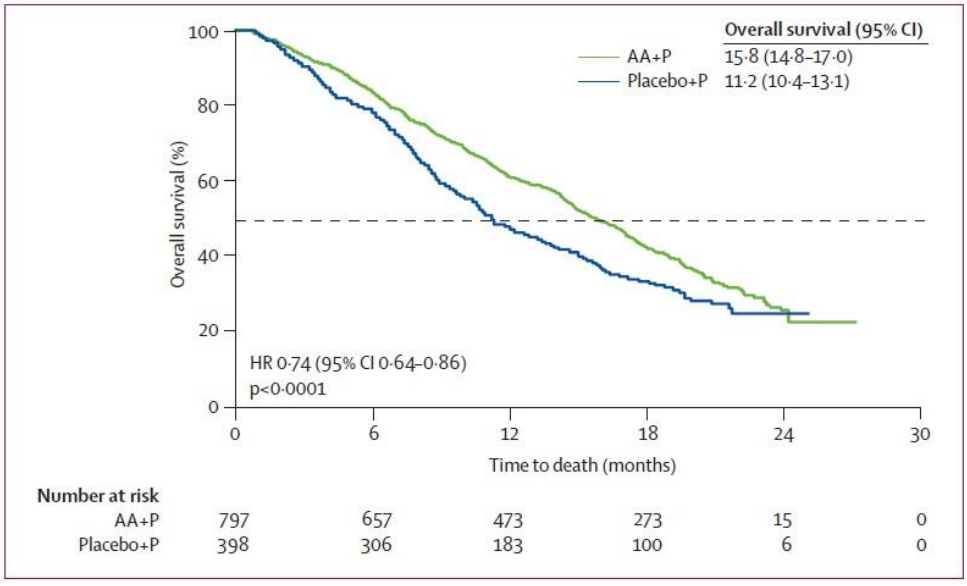


Figure 16: From (Fizazi et al., 2012) Median overall survival was 15·8 months (95% CI 14·8–17·0) in the abiraterone group compared with 11·2 months (10·4–13·1) in the placebo group (hazard ratio [HR] 0·74, 95% CI 0·64–0·86; p<0·0001 (HR=hazard ratio. AA=abiraterone acetate. P=prednisone)

ECOG PERFORMANCE STATUS	
Grade	**ECOG**
0	Fully active, able to carry on all pre-disease performance without restriction
1	Restricted in physically strenuous activity but ambulatory and able to carry out work of a light or sedentary nature, e.g., light house work, office work
2	Ambulatory and capable of all selfcare but unable to carry out any work activities. Up and about more than 50% of waking hours
3	Capable of only limited selfcare, confined to bed or chair more than 50% of waking hours
4	Completely disabled. Cannot carry on any selfcare. Totally confined to bed or chair
5	Dead

Figure 17: From (Oken et al., 1982) Eastern Cooperative Oncology Group Performance Status

This was supported by statistically significant secondary endpoints in favor of abiraterone, including median time to PSA progression (8.5 months versus 6.6 months), median radiologic progression-free survival (rPFS; 5.6 months versus 3.6 months), and proportion of patients with ≥50% PSA decline (29.5% versus 5.5%). The impact of abiraterone on OS was observed across all subgroups, including patients who had received one (17.1 versus 11.7 months) or two prior chemotherapy regimens (14.2 versus 10.4 months) (Figure 18). Notably, patients on abiraterone with a performance status of two had worse outcomes, with a median survival of 7.3 months versus those with performance status of of zero to one receiving abiraterone with median survival of 15.3 months(Figure 19)(Fizazi et al., 2012).

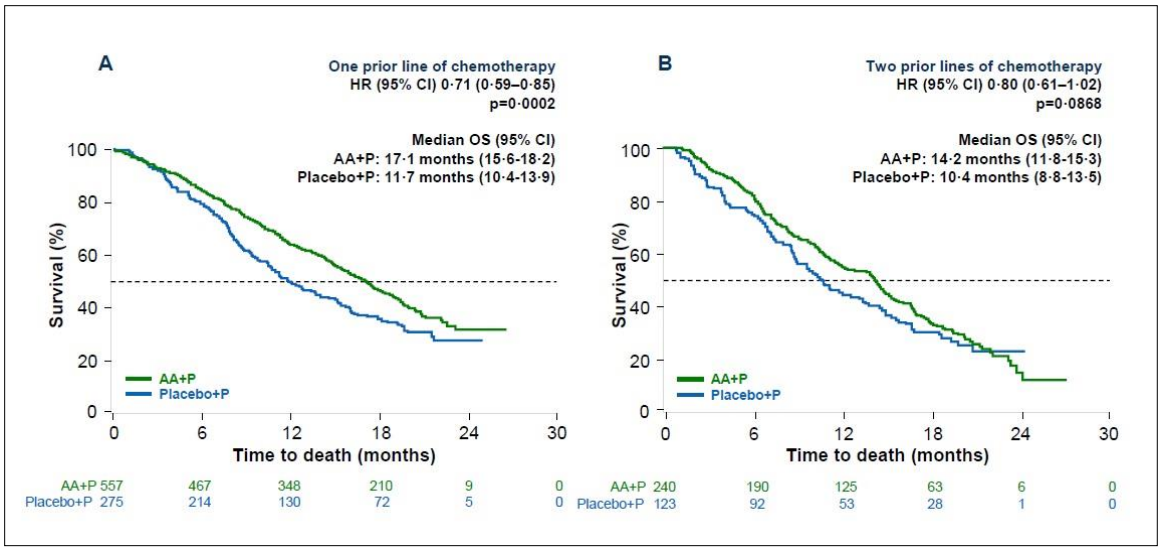


Figure 18 : From (Fizazi et al., 2012)9Survival benefit observed with AA for subgroups with one (A) or two (B) prior lines of chemotherapy at study entry HR=hazard ratio; CI=confidence interval; OS=overall survival; AA=abiraterone acetate.

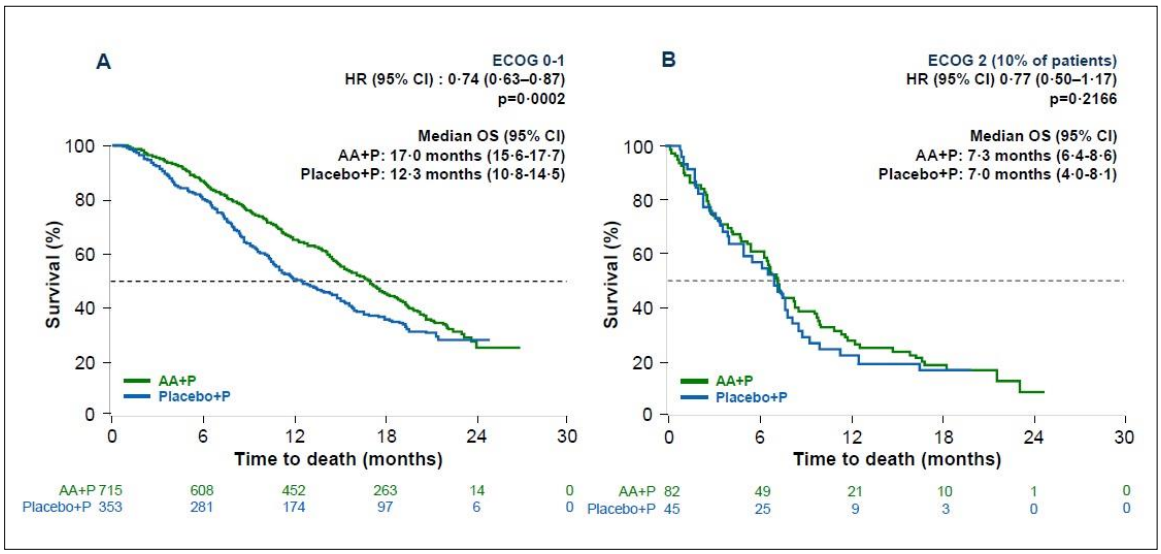


Figure 19 : From (Fizazi et al., 2012)Survival by baseline ECOG status favors AA for ECOG 0–1 (A), but not for ECOG 2 (B) ECOG= Eastern Cooperative Oncology Group; HR=hazard ratio; CI=confidence interval; OS=overall survival; AA=abiraterone acetate

Although visceral disease was associated with a poorer prognosis, an exploratory study found the absolute benefit in OS from abiraterone to be similar in those with and without visceral disease (8.3–12.9 months in those with visceral disease and 12.3–17.3 months in those without) (Goodman et al., 2014).

Exploratory analysis of COU-AA-301 showed that abiraterone significantly increased the number of patients reporting an improvement in fatigue intensity (58.1% versus 40.3%; *P*=0.0001) as well as the time to fatigue palliation (median 59 days versus 194 days; *P*=0.0155) (Figure 20)(Sternberg et al., 2013). Abiraterone significantly increased the number of patients reporting palliation of pain (45% versus 28.8%; *P*=0.0005), as well as faster palliation (median time to palliation 5.6 months versus 13.7 months; *P*=0.0018). Significant longer median time to occurrence of first skeletal-related event was observed in abiraterone treated patients (25 months versus 20.3 months; *P*=0.0001). Skeletal related events included pathologic fracture, spinal cord compression, or palliative surgery or radiation to bone(Sternberg et al., 2013)(Mostaghel, 2014a).

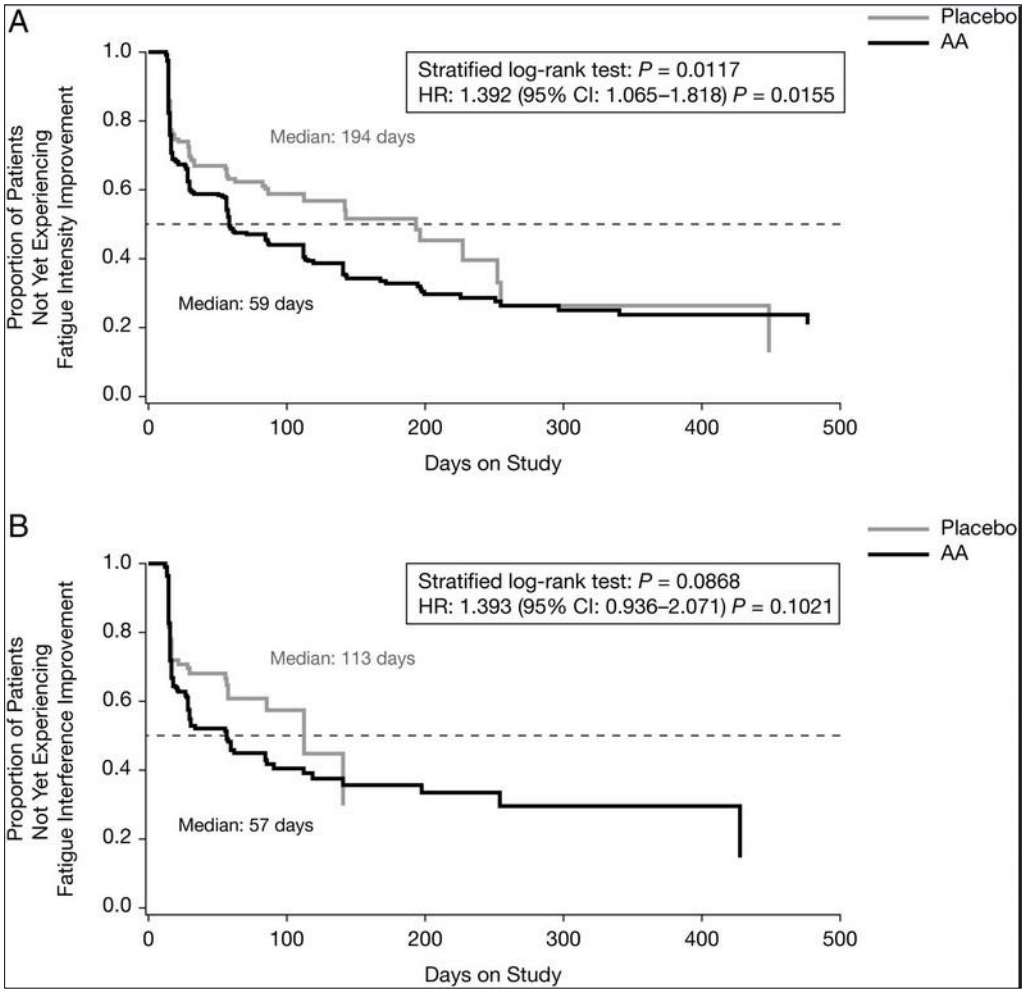
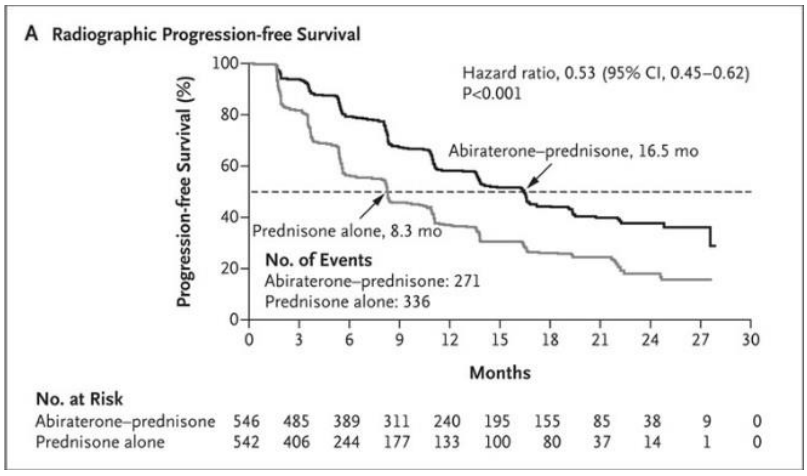


Figure 20: From (Sternberg et al., 2013)Time to symptomatic improvement: (A) fatigue intensity; (B) fatigue interference.

COU-AA-302

In this double blind study, the efficacy of abiraterone was assessed in CRPC patients who had not received previous chemotherapy. 1,088 men with asymptomatic or minimally symptomatic bone and lymph node (but not visceral) metastatic CRPC were randomized 1:1 to abiraterone (100mg)/prednisone (5mg twicw daily) (n=546) or placebo/prednisone (n=542), with co-primary endpoints of rPFS and OS. The median PSA was ~40 ng/dL, about 30% of men were more than 75 years, and approximately 50%had bone-only metastatic disease.

At a median follow-up of 22.2 months, rPFS was 8.3 months in the placebo arm and 16.5 months in men receiving abiraterone (HR 0.53; *P*<0.001)(Figure 21a). The median overall survival was not reached in the abiraterone arm, but it was comparatively less in placebo (only prednisone) arm with OS of 27.2 months. This accounted for the 25% decrease in death risk in abiraterone-prednisone group. Thus abiraterone offered improvement in overall survival (Figure 21) (Ryan et al., 2013).An updated analysis conducted at median survival of 27.1 months favored abiraterone with OS at 30.1 months in the placebo arm versus 35.3 months in the abiraterone arm (HR 0.79; *P*=0.015)(Rathkopf et al., 2013)(Mostaghel, 2014a).



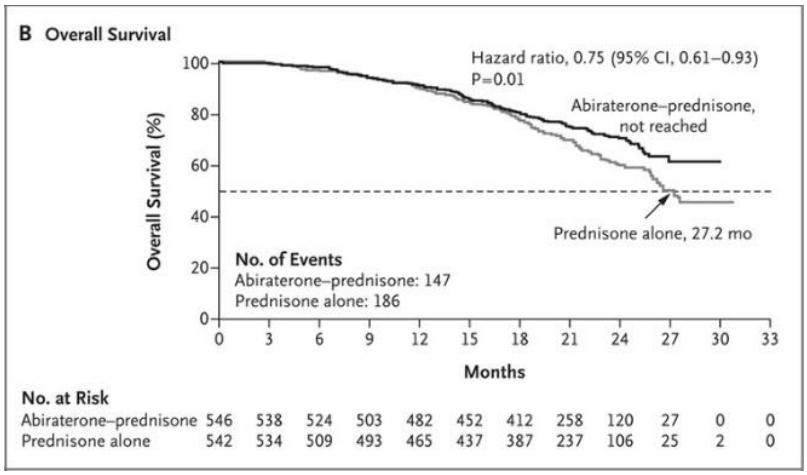


Figure 21: From (Ryan,C.J. 2013) Kaplan–Meier Estimates of Radiographic Progression-free Survival, Overall Survival, and Subgroup Analyses at the Second Interim Analysis.
Panel A shows data for radiographic progression free survival on the basis of investigator review, and Panels B show data for overall survival. The dashed line in Panels A and B indicates the median. All analyses were performed with the use of a stratified log-rank test according to the baseline score on the Eastern Cooperative Oncology Group (ECOG) scale (a performance status grade of 0 indicates asymptomatic, and 1 restricted in strenuous activity but ambulatory).
.

In this study as well, all secondary endpoints were statistically significant in favor of abiraterone, with decrease in use of opiates by patients on abiraterone(median time to opiate use not reached) versus prednisone alone treated patients (median time to opiate use 23.7 months), time to initiation of chemotherapy (25.2 months versus 16.8 months), time for performance status decline (12.3 months versus 10.9 months), time to PSA progression (11.1 months versus 5.6 months), and proportion of patients with ≥50% PSA response (62% versus 24%). The impact of abiraterone on rPFS was observed across all subgroups(Figure 22)(Ryan et al., 2013). While this study did not include patients with visceral disease or moderate to severe pain, the exploratory analyses of these subpopulations in the post-chemotherapy setting discussed above suggest these patients are likely to benefit as well (Mostaghel, 2014a).

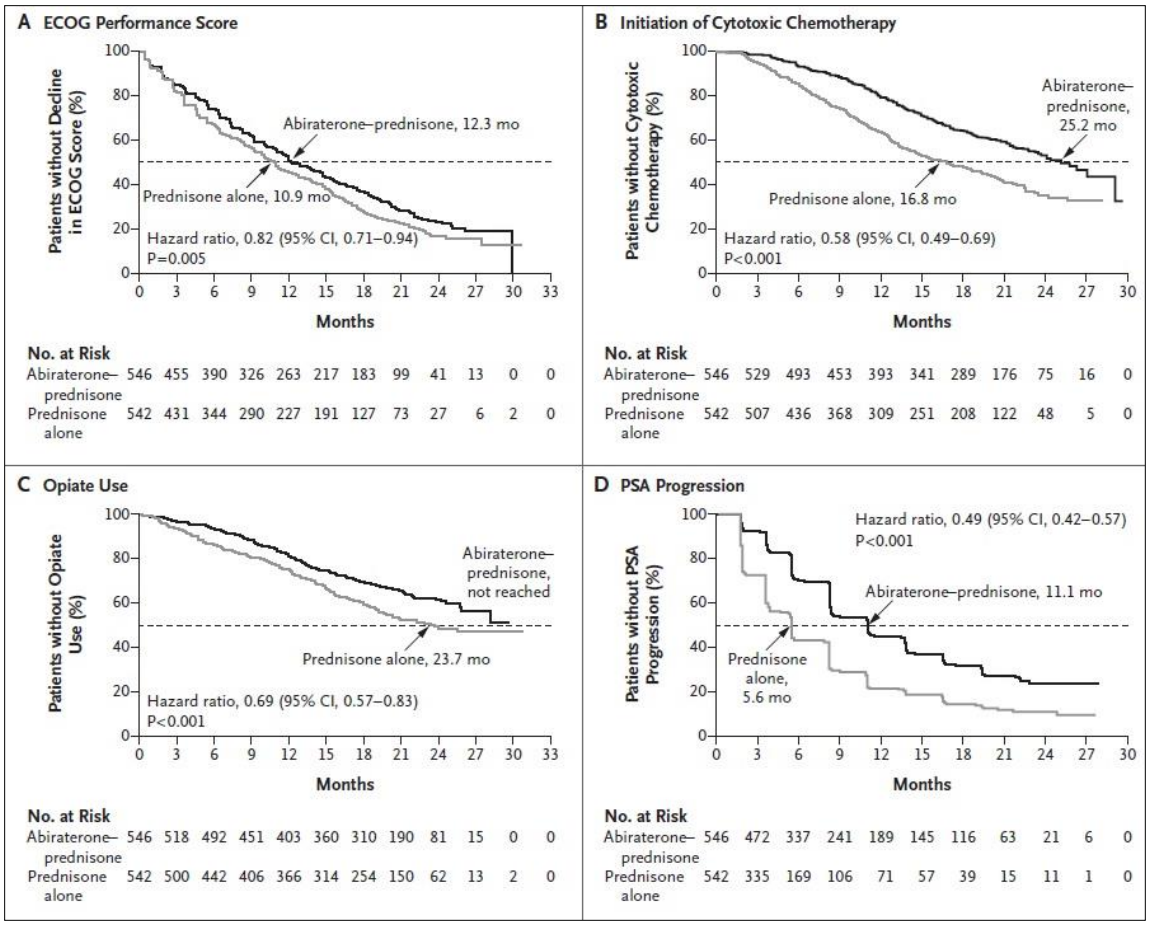


Figure 22: From (Ryan et al., 2013)**Secondary Efficacy End Points.**
Shown are the time until a decline in the Eastern Cooperative Oncology Group (ECOG) score by one point or more (Panel A), the time until the initiation of cytotoxic chemotherapy (Panel B), the time until the use of opiates for pain from prostate cancer (Panel C), and the time until prostate-specific antigen (PSA) progression according to Prostate Cancer Clinical Trials Working Group 2 criteria2 (Panel D). The dashed line indicates the median. All analyses were performed with the use of a stratified log-rank test according to the baseline ECOG score.

Tolerability:

Abiraterone is generally well tolerated, with 13%abiraterone-treated patients in COU-AA-301 and 19% in COU-AA-302 discontinuing therapy for adverse effects versus 18%of placebo-treated patients in COU-AA-301 and 23% in COU-AA-302respectively. The major adverse events in these groups were fatigue, back pain, nausea, constipation, bone pain, and arthralgia, in the range of 25%–30%. The incidence of urinary tract infection was statistically higher in abiraterone-treated patients (12% versus 7% in placebo; *P*=0.02).(Fizazi et al., 2012)(Logothetis et al., 2012)(Ryan et al., 2013)

Mineralocorticoid and electrolyte effects:

Inhibition of CYP17A results in elevation of ACTH as mentioned previously by decreasing glucocorticoids downstream of CYP17A. This decrease in cortisol inhibits the negative feedback on hypothalamus-pituitary axis leading to rise of ACTH levels. This further leads to enhancement of adrenal steroidogenesis causing increased aldosterone and corticosterone production (Figure 12). The symptoms associated with mineralocorticoid excess like fluid retention, hypertension, hypokalemia; occurred in 50-80% patients of Phase I/II trials versus those in Phase III trials who showed marked reduction in these symptoms- fluid retention (~33% versus 22%–24% in placebo), hypertension (~10% versus 8% in placebo), and hypokalemia (~18% versus 9% in placebo). Dexamethasone which blocks the rise in ACTH secretion can be used at a dose of 0.5mg daily. Although it lacks mineralocorticoid effects, its addition might lead to rare incidence of orthostatic hypotension(Attard et al., 2008)(Ryan et al., 2010).

Hepatotoxicity:

Grade III or IV hepatic transaminase abnormalities (five times the upper limit of normal ULN) occurred in approximately 4% of patients in the Phase III studies, usually within the first 3 months of starting treatment. This occurred more commonly in men with elevated baseline levels of alanine transaminase or aspartate transaminase. It is thus recommended that serum transaminases should be measured at baseline. Transaminases in patients with normal levels should be checked every 2 weeks for the first 3 months of therapy followed by monthly checks. Mild hepatic impairment does not require dose adjustments. On the other hand, for moderate hepatic impairment, abiraterone should be started at 250 mg daily, with weekly checks of transaminases for the first month, then every 2 weeks for the following 2 months, and then monthly.

Abiraterone should be held if aspartate transaminase or alanine transaminase levels rise more than five times the ULN or bilirubin rises above three times the ULN. It should be discontinued if the patient had moderate hepatic impairment at baseline, but in patients with normal hepatic function at baseline it can be restarted at 750 mg daily after the decline of liver function tests and total bilirubin to less than 2.5 times and 1.5 times the ULN, respectively. If hepatotoxicity recurs, a further dose reduction to 500 mg can be

attempted (once levels have fallen below the thresholds given above), however the drug should be discontinued with recurrence of hepatotoxicity at the 500 mg dose.

Cardiotoxicity:

The overall incidence of adverse cardiac effects was not statistically increased by abiraterone in COU-001 (13% versus 11% in placebo), although the frequency of cardiac failure was higher in the abiraterone group (2.1% versus 0.7% in placebo). The most frequently reported cardiac events were Grade I and II tachycardia and Grade III or lower atrial fibrillation. A retrospective study of 51 metastatic CRPC patients with at least one cardiac comorbidity and/or controlled risk factor including hypertension (41%), hyperglycemia (30%), dyslipidemia (18%), cardiac ischemia (12%), stroke (9%), or arrhythmias (6%) reported no cardiac events or variation in left ventricular ejection fraction over 6–12 months of follow-up(Procopio et al., 2013). However, as patients with left ventricular ejection fraction ≤50% were excluded from the Phase III studies, electrocardiogram and echocardiography offer pretreatment assessment and optimization of cardiac status which may warrant consideration in elderly patients with reduced cardiac function. A significant effect of abiraterone on the QT/QTc interval in patients with CRPC was not observed(Tolcher et al., 2012).

B. Androgen Receptor Blocker: Enzalutamide (Xtandi)

Previous studies have shown that residual androgen levels in patients with prostate cancer may be amplified due to factors like mutation of AR, overexpression of AR, alterations in levels of cofactors etc(Debes and Tindall, 2004b)(Aggarwal and Ryan, 2011). This overexpression of AR was linked to presence of functional Ligand Binding Domain (LBD) which conferred resistance to anti-androgens(Chen et al., 2004).The first generation of available androgen-receptor antagonists such as bicalutamide or flutamide have agonist property in cells expressing high levels of AR. They also had low binding affinity and partial agonism(Tran et al., 2009). Hence, there was a need to design a drug which could overcome these deficiencies. In the process to search for improved anti androgens, RU59063 was selected as a starting chemical scaffold based on its high affinity to AR(Van Dort, Robins, and Wayburn, 2000) (Figure 23). Extensive studies were done on this chemical to generate the diarylthiohydantoinsRD162 and MDV3100 (later known as Enzalutamide) as the lead compounds for further biological studies(Tran et al., 2009).

Structure:

Enzalutamide has a chemical designation as 4-{3-[4-cyano-3-(trifluoromethyl)phenyl]-5,5-dimethyl-4-oxo-2-sulfanylideneimidazolidin-1-yl}-2-fluoro-N-methylbenzamide.Its molecular formula is C21H16F4N4O2S, with a molecular weight of 464.44. Enzalutamide is a white crystalline non hygroscopic solid that is practically insoluble in water. Enzalutamide is formulated in liquid-filled soft gelatin capsules marketed as XTANDI. Each capsule contains 40 mg of enzalutamide as a solution in caprylocaproyl polyoxylglycerides(Ning et al., 2013).

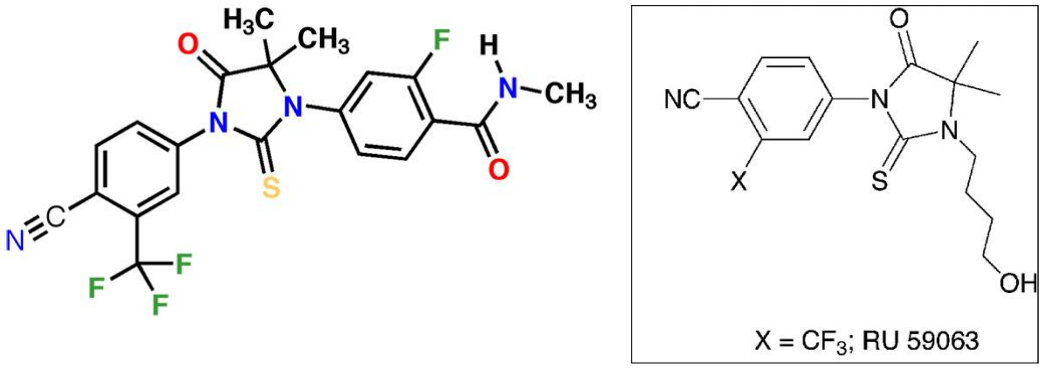


Figure 23: Left: Structure of Xtandi(Enzalutamide) from (Kable Intelligence Limited, 2015) and right:structure of RU59063 from (Van Dort et al., 2000)

A structure-activity relationship study was conducted on many analogous diaryl thiohydantoins that led to the choice of **Enzalutamide**as a clinical candidate for the treatment of castration-resistant prostate cancer. Among the candidates, the pharmacokinetic properties of **MDV3100 (Enzalutamide)**led to its choice as the clinical candidate(Jung et al., 2010).

Mechanism of Action:

MDV3100 is a novel androgen-receptor antagonist selected for activity in both in-vitro and in-vivo model systems of prostate cancer with over expressed androgen receptor(Tran et al., 2009)(Scher et al., 2010). *Invitro* study was done using LNCap/AR human prostate cancer cells. It was observed that MDV3100 has a five to eight fold higher affinity for androgen receptor than bicalutamide when evaluated using an 18-fluoro-deoxyglucose-dihydrotestosterone scan to measure relative AR binding affinity in a competition assay. Enzalutamide antagonized induction of PSA and transmembrane serine protease 2, and lacked intrinsic agonist activity. Thus, when compared to bicalutamide, MDV3100 is a pure antagonist, with no detectable agonist effects in LNCaP/AR prostate cells, which over express androgen receptor.MDV3100 compared to bicalutamide suppresses growth, induces apoptosis, impairs androgen binding to the receptor, nuclear translocation, DNA binding, and co-activator recruitment (Figure 24)(Tran et al., 2009).

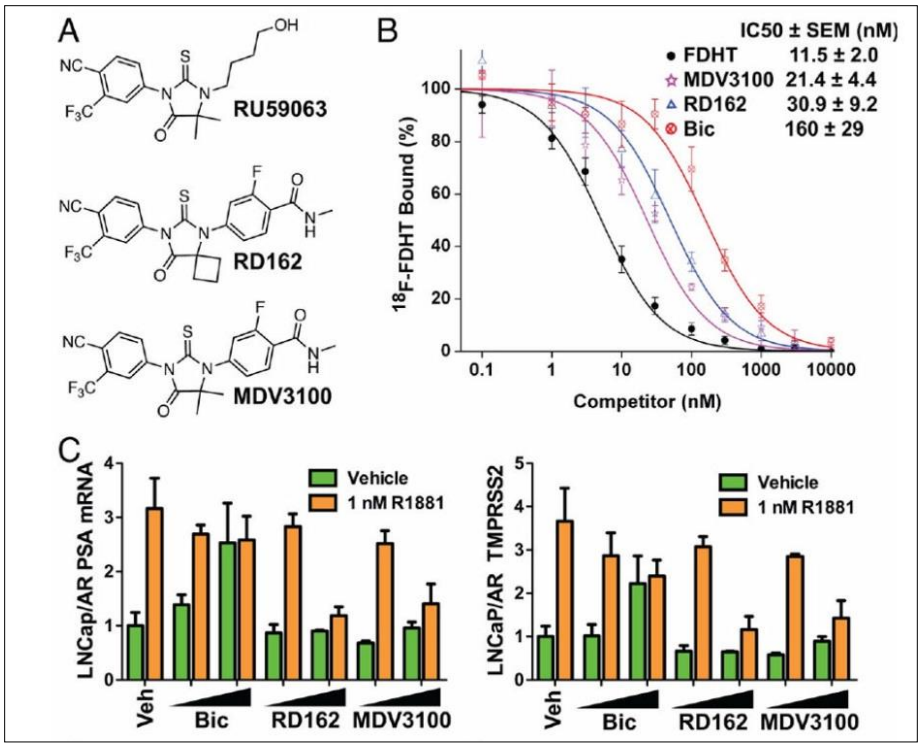


Figure 24: From (Tran et al., 2009) - Effect of RD162 and MDV3100 in human prostate cancer cells *in vitro*. (**A**) Chemical structures of the parent arylthiohydantoin scaffold compound RU59063 and the novel AR antagonists RD162 and MDV3100. (**B**) Representative competition binding curve showing inhibition of 18F-FDHT equilibrium binding to AR by FDHT, RD162, MDV3100 and bicalutamide (Bic) in LNCaP/AR cells.(**C**) qRT-PCR analysis of the AR-dependent genes PSA and TMPRSS2 in LNCaP/AR cells cultured in androgen-depleted media with 5% charcoal-stripped serum (CSS). Cells were treated for 8 hours with or without 1nM of the synthetic androgen R1881 combined with DMSO (Veh), bicalutamide (Bic, 1 and 10 μM), RD162 (1 and 10 μM) and MDV3100 (1 and 10 μM) (normalized to actin mRNA, Mean ± SD, n=3).

The drug also induces regression of established LNCaP/AR xenograft tumours growing in castrated male mice—a model in which bicalutamide treatment only slows tumour growth.. The regression is associated with continued evidence of apoptosis up to 25 days after the start of treatment (figure 25) (Tran et al., 2009). Based on these results, MDV3100 was selected for clinical development in the Prostate Cancer Clinical Trials Consortium (Morris et al., 2009). An overview of Enzalutamide mechanism of action is depicted in figure 26(Patel NK et al. 2014).

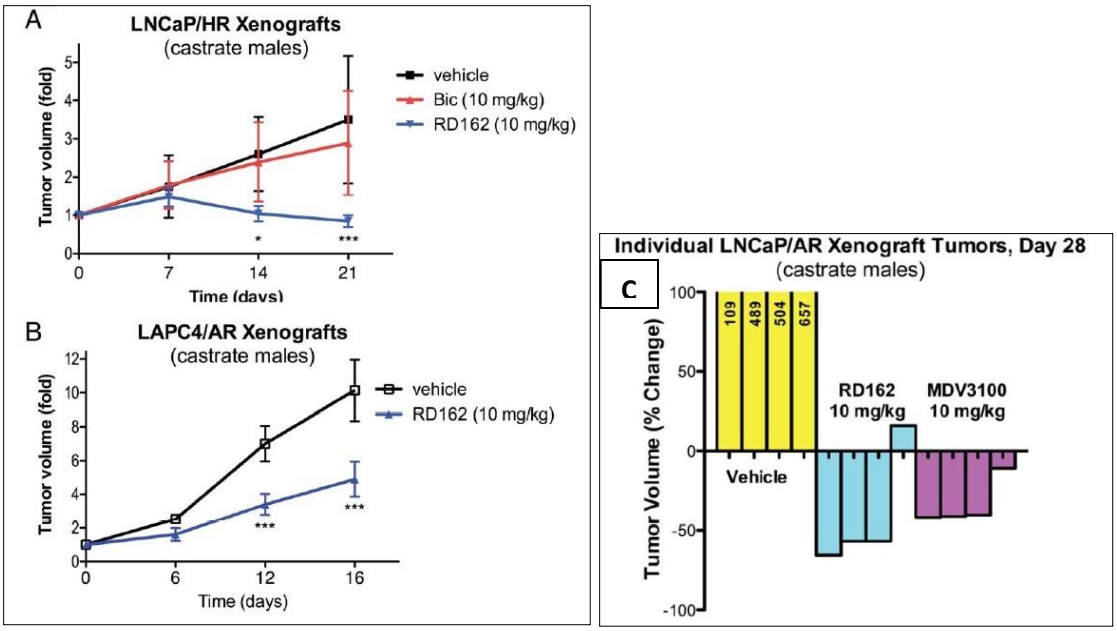


Figure 25: From [7(Tran et al., 2009)9]
A,B: In vivo activity of RD162 in other castration-resistant prostate cancer models
(A) Castration-resistant LNCaP cells were derived from parental LNCaP cells by serial passage in castrate male mice.Castrate male mice bearing LNCaP/HR xenografts were treated by daily oral gavage for 21 days with vehicle or RD162 (10 mg/kg). * $P < 0.05$, *** = $P < 0.001$. (B) Castrate male mice bearing LAPC4/AR xenografts (*1*) were treated by daily oral gavage for 16 days with vehicle or RD162 (10 mg/kg).
C : Tumor regression following MDV3100 or RD162 treatment in the LNCaP/AR xenograft
Model. Castrate male mice bearing LNCaP/AR xenografts (n=4 per treatment group) were treated by daily oralgavage with vehicle, RD162 or MDV3100 at 10 mg/kg.

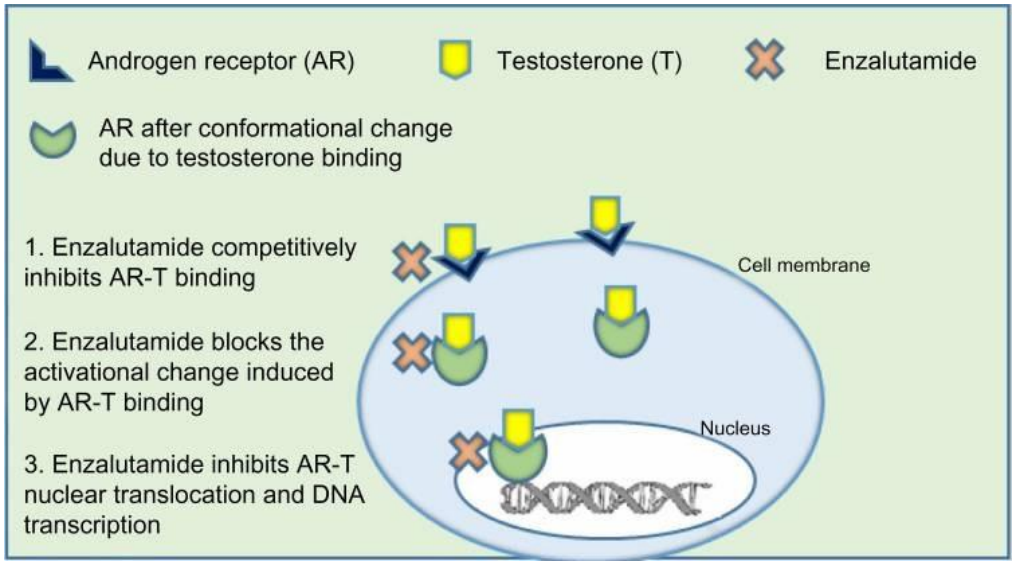


Figure 26: Mechanism of Action of Enzalutamide (Patel NK et al., 2014)

Development and Drug Interactions:

Enzalutamide was jointly developed and manufactured by Medivation and Astellas Pharma. The US Food and Drug Administration(FDA) accepted the new drug application for Xtandi in July 2012. The FDA approved the drug in August 2012, for the treatment of mCRPC patients who were treated previously with docetaxel-based chemotherapy. Three months later, in November 2012,the drug was reviewed for manufacturing purpose. The marketing authorisation application of the drug has also been accepted for review by the European Medicines Agency (EMA) (Kable Intelligence Limited, 2015).

Enzalutamide may interact with known inducers of CYP2C8 or CYP3A4 and hence the co-administration of these drugs should be monitored, although formal drug interactions studies for evaluation of these effects have not been conducted yet. In vivo, the sum of enzalutamide and N-desmethyl enzalutamide exposure was increased by 2.2-fold and 1.3-fold when it was coadministered with gemfibrozil (strong CYP2C8 inhibitor) or itraconazole (strong CYP3A4) inhibitor), respectively, suggesting the need for avoidance of such coadministration(El-Amm et al., 2013).

Clinical Trials:

Enzalutamide was more potent than earlier generation of antiandrogens including flut-amide, nilutamide, and bicalutamide as shown in early preclinical studies (Figure 24)[79]. As mentioned previously, Enzalutamide showed greater affinity for androgen receptor compared to bicalutamide and also acted as a pure antagonist at AR. These preclinical results supported the need for Phase I/II trials in humans (El-Amm et al., 2013).

Phase I/II trials:

Phase I/II trial was conducted to assess efficacy, safety and tolerability profile of MDV3100 and to establish the maximum tolerated dose. The trial was registered with ClinicalTrials.gov, number NCT00510718(Scher et al., 2010).140 patients with CRPC were enrolled at multiple centers in US to receive enzalutamide orally at doses ranging from 30 mg to 600 mg daily as follows 30 mg (n=3), 60 mg (27), 150 mg (28), 240 mg (29), 360 mg (28), 480 mg (22), and 600 mg (3).The vast majority of the patients (78%)

included in this trial had metastatic disease. Around 44% of the patients had not received any previous therapy, 30% had previously undergone surgery and 26% had previously received definitive radiation therapy. Around half of the patients had previously received chemotherapy and over 75% of the patients had previously received at least two lines of hormonal therapy. 240mg daily was determined as the maximum tolerated dose and higher dosages did not offer any additional benefit. Antitumor activity was observed at all tested dosages. The median time to radiological progression was 47 weeks for all patients. This median time was more prolonged with it being >60 weeks in the chemotherapy-naïve group compared to 29 weeks in the chemotherapy pretreated group. Some of the antitumor effects seen included decreases in serum prostate-specific antigen of 50% or more in 78 (56%) patients, responses in soft tissue in 13 (22%) of 59 patients, stabilised bone disease in 61 (56%) of109 patients, and conversion to favourable circulating tumor cell counts from unfavourable in 25 (49%) of the51 patients., indicating an overall positive effect on this adverse prognostic group of patients. The major side effects included headache, hot flashes, and fatigue. Fatigue was dose-dependent and occurred in 11% of the patients. Three patients who were receiving the 360 mg dosage or higher developed seizure, and two of the patients were on medications that lowered the seizure threshold. An updated analysis showed that 18 of the enrolled patients remained in the study, with a median time on therapy of 131 weeks(Higano et al., 2011)(El-Amm et al., 2013).The median time to PSA progression (as assessed by the Prostate Cancer Working Group 2)was 41 weeks and 20 weeks in the chemotherapy-naïve and the chemotherapy-pretreated groups, respectively. The median radiographic PFS was 56 weeks and 24 weeks in the chemotherapy-naïve and the chemotherapy-pretreated group, respectively (Figure 27)(El-Amm et al., 2013)(Scher et al., 2010).Thus in Phase I/II study conducted by the Prostate Cancer Clinical Trials Consortium, it was confirmed that enzalutamide had significant anti-tumor activity regardless of previous chemotherapy status(Scher et al., 2012).

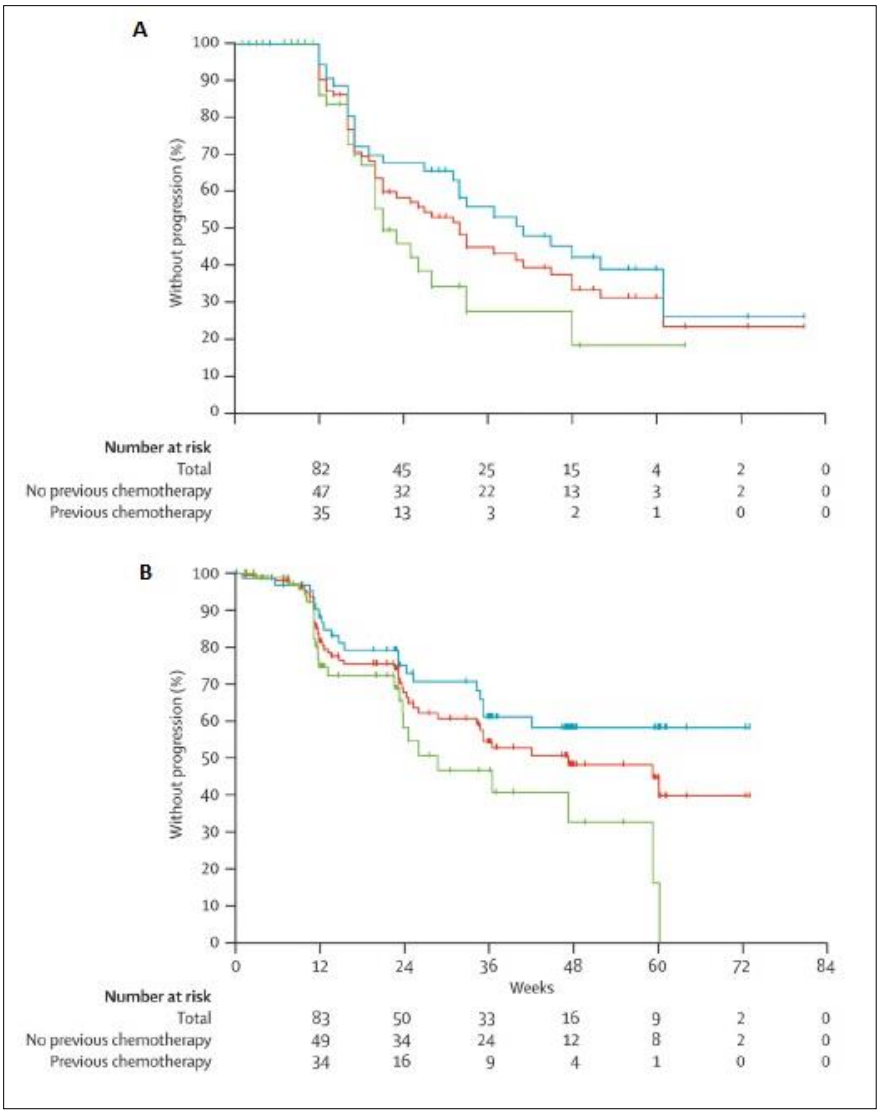


Figure 27: From (Scher et al., 2010)Time to progression (A) PSA progression using the PCWG criterion of a 25% or greater rise from nadir (B) Imaging.

Phase III Trial:

AFFIRM trial:

The Phase III AFFIRM trial (A Study Evaluating the Efficacy and Safety of the Investigational Drug MDV3100 - Funded by Medivation and Astellas Pharma Global Development; AFFIRM ClinicalTrials.gov number, **NCT00974311**) was an international double-blind placebo controlled trial in men with mCRPC who have failed prior docetaxel-containing chemotherapy regimens(Scher et al., 2012)(El-Amm et al., 2013).A total of 1,199 men with mCRPC from 166 sites were randomized in a 2:1 manner to

receive either enzalutamide 160 mg daily (n = 800) or placebo (n = 399). The primary endpoint of the trial was OS (overall survival) and the secondary endpoints included radiographic PFS, time to PSA progression, quality of life, as well as time to the first skeletal-related event (SRE). Patients were eligible to be enrolled in the trial with post docetaxel disease progression, presence of adequate organ function, an Eastern Cooperative Oncology group (ECOG) performance status of zero to two. Patients on placebo were allowed to cross-over to receive enzalutamide based on recommendation by the Data and Safety Monitoring Committee. Despite this cross-over, median follow-up of 14 months showed that there was significant improvement in the median OS of patients in the enzalutamide arm versus the placebo arm as shown in figure 28A {18.4 months versus 13.6 months, respectively; hazard ratio (HR), 0.63; 95% confidence interval (CI), 0.53–0.75; P <0.0001}. This 4.8-month difference in OS indicated reduction in the risk of death due to any cause by 37% in the enzalutamide arm. An interim analysis after a total of 520 deaths showed that enzalutamide was superior to placebo in all the examined secondary endpoints, even in poor-risk. The group of patients who did not appear to benefit from enzalutamide was the one that included patients who received two or more prior chemotherapy regimens(Scher et al., 2012)(El-Amm et al., 2013).

The superiority of enzalutamide over placebo was shown for all secondary end points, including PSA-level response rate (54% vs. 2%, P<0.001), soft-tissue response rate (29% vs. 4%, P<0.001), FACT-P quality-of-life response (43% vs. 18%, P<0.001), the time to PSA progression (8.3 vs. 3.0 months; hazard ratio, 0.25; P<0.001) (Fig. 28B), radiographic progression-free survival (8.3 vs. 2.9 months; hazard ratio, 0.40; P<0.001) (Fig. 28C), and the time to the first skeletal-related event (16.7 vs. 13.3 months; hazard ratio, 0.69; P<0.001) (Scher et al., 2012).This study concluded that enzalutamide significantly prolonged the survival in men with mCRPC after chemotherapy.

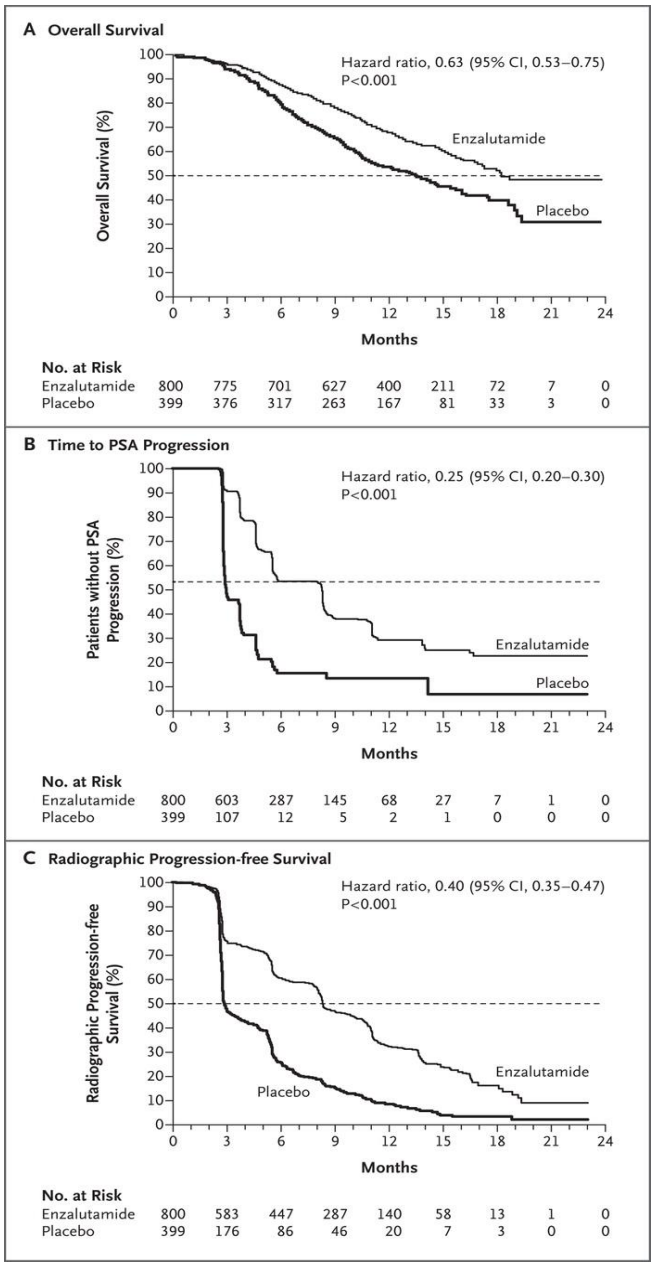


Figure 28: Kaplan–Meier Estimates of Primary and Secondary End Points in the Intention-to-Treat Population. Shown are data for overall survival, the primary end point (Panel A), and for two secondary end points, the time to prostate-specific antigen (PSA) progression (Panel B) and radiographic progression-free survival (Panel C), in the enzalutamide group, as compared with the placebo group. CI denotes confidence interval. From (Scher,H.I. 2012)

PREVAIL trial:

The PREVAIL study was a multinational, doubleblind, randomized, placebo-controlled, phase 3trial of enzalutamide (Funded by Medivation and Astellas Pharma; PREVAIL ClinicalTrials.gov number, **NCT01212991**.Patients were randomly assigned to receive either enzalutamide (at a dose of 160 mg) or placebo once daily. The coprimary end points were radiographic progression-free survival and overall survival(Beer et al., 2014).

As with the previous AFFIRM trial, this study was also halted at a planned interim analysis. The rate of radiographic progression-free survival at 12 months was 65% among patients treated with enzalutamide, as compared with 14% among patients receiving placebo (Figure 29A) (81% risk reduction; hazard ratio in the enzalutamide group, 0.19; 95% confidence interval [CI], 0.15 to 0.23; P<0.001). A total of 626 patients (72%) in the enzalutamide group, as compared with 532 patients (63%) in the placebo group, were alive at the data-cutoff date (Figure 29B)(29% reduction in the risk of death; hazard ratio, 0.71; 95% CI, 0.60 to 0.84; P<0.001). All secondary endpoints showed significant benefit with enzalutamide. These benefited secondary end points include the time until the initiation of cytotoxic chemotherapy (Figure 30A) (hazard ratio, 0.35), the time until the first skeletal-related event (hazard ratio, 0.72), a complete or partial soft-tissue response (59% vs. 5%), the time until prostate-specific antigen (PSA) progression (Figure 30B)(hazard ratio, 0.17), and a rate of decline of at least 50% in PSA (78% vs. 3%) (P<0.001 for all comparisons). Fatigue and hypertension were the most common clinically relevant adverse events associated with enzalutamide treatment. This study concluded that enzalutamide is an orally active drug with minimal side effects, when given to men with modest symptoms or asymptomatic mCRPC with no previous chemotherapy, it significantly delayed radiographic disease progression or death, the need for cytotoxic chemotherapy, and the deterioration in quality of life and significantly improved overall survival(Beer et al., 2014).

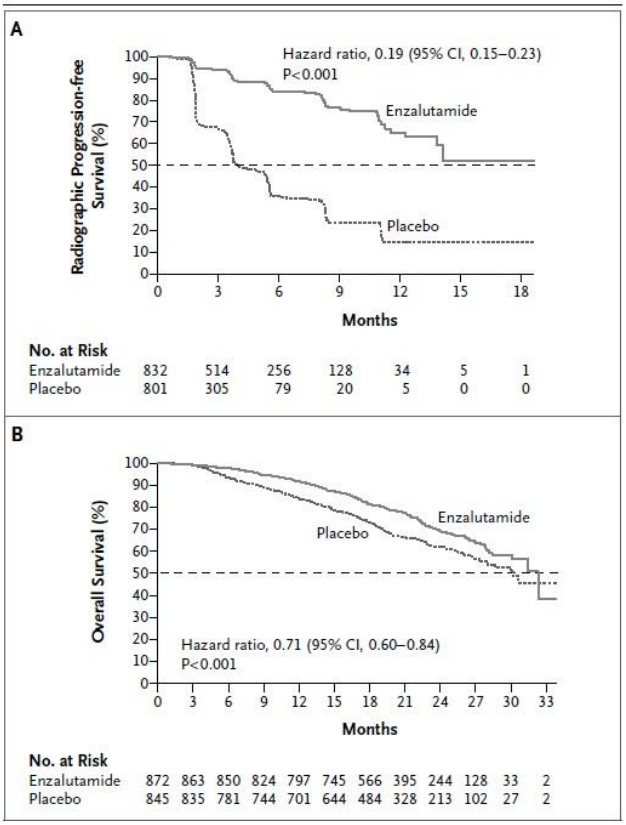


Figure 29. Kaplan–Meier Estimates of Radiographic Progression-free Survival and Overall Survival**.**
Shown are data for the coprimary end points of radiographic progressionfree survival (Panel A) and overall survival (Panel B). The dashed horizontal lines indicate medians. Hazard ratios are based on unstratified Cox regression models with treatment as the only covariate, with values of less than 1.00 favoring enzalutamide. From (Beer,et al. 2014)

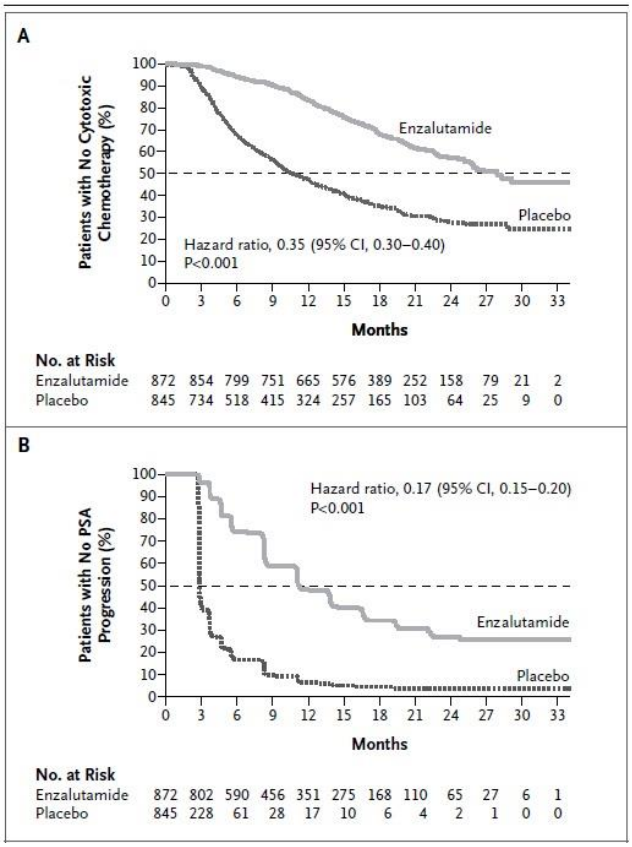


Figure 30. Kaplan–Meier Estimates for the Times until the Initiation of Cytotoxic Chemotherapy and an Increased Level of Prostate-Specific Antigen.
Shown are secondary efficacy end points that include the time until the initiation of cytotoxic chemotherapy (Panel A) and the time until an increased level of prostate-specific antigen (PSA) (Panel B). The horizontal dashed lines indicate medians. Hazard ratios are based on unstratified Cox regression models with treatment as the only covariate, with values of less than 1.00 favoring enzalutamide. From (Beer,et al. 2014)

Combinational Therapy:

Secondary line therapy resistance:

Metastatic castration resistant prostate cancer patients develop resistance to the above discussed novel anti-androgen approaches over the years. The mechanism for this resistance is not yet clearly understood but several possibilities have been proposed and tested in preclinical models as well as clinical studies(Ammannagar and George, 2015).

In one preclinical prostate cancer model study, Arora et al. proposed that enzalutamide resistance was mediated by glucocorticoid receptors(GR). The investigators were able to showan upregulation of GR in prostate cancer cell lines and that dexamethasone reversed enzalutamide- induced growth inhibition. A positive correlation was reported between GR expression in patient-derived prostate cancer specimens and clinical response to enzalutamide (Arora et al., 2013)(Ammannagar and George, 2015).

Abiraterone strongly inhibits CYP17A enzyme. Despite this action, resistance to abiraterone may develop due to continuous intratumoral steroidogenesis via denovo synthesis within tumor or through uptake and conversion of adrenal androgens (Li et al., 2012).

AR-V7: *Androgen receptor variant 7*

AR-V7 is an AR isoform encoded by splice variant 7. This spliced variant encodes a truncated AR protein that lacks the C-terminal LBD, but remains constitutively active as a transcription factor and is capable of promoting activation of some target genes via its activation function-1 domain (**Figure 31**). The expression of AR-V7 is increased by nearly 20-fold in CRPC tumor cells in certain patients. Enzalutamide interacts with the LBD of AR, and hence the AR-V7 without the LBD may cause enzalutamide resistance. Moreover, abiraterone causes reduction in ligand levels, hence it becomes apparent that abiraterone will not work in the presence of the ligand-independent AR-V7 protein. A prospective study showed that the detection of AR-V7 may be associated with primary and acquired resistance to enzalutamide and abiraterone. AR-V7 positive patients showed lower PSA response rates, shorter progression free survival and decreased overall survival, compared to AR-V7 negative patients. Thus, patients with CRPC patients who

areAR-V7-positive in circulating tumor cells(CTC) sample should not be offered AR-targeting drugs but instead alternative treatments modalities should opted.(Haile and Sadar, 2011)(Antonarakis et al., 2014)

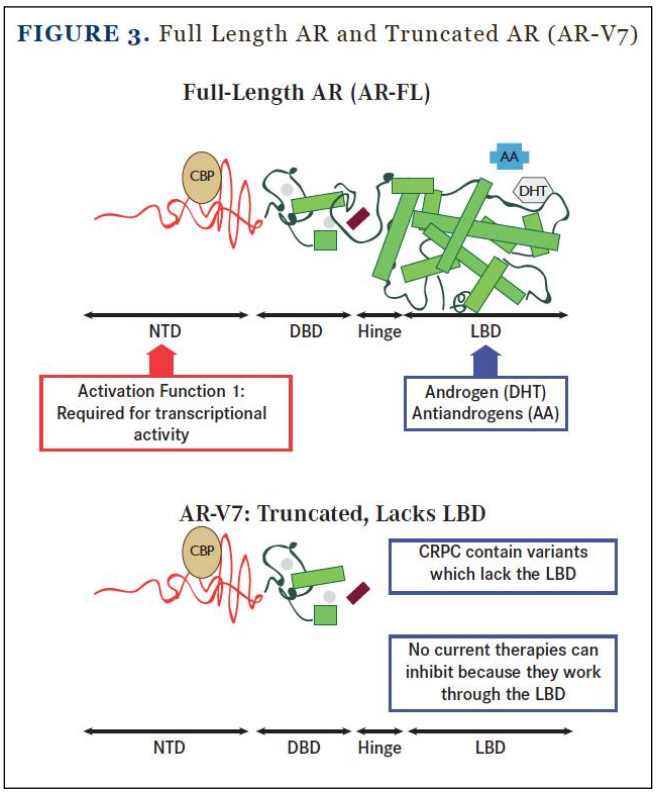


Figure 31: Full length AR and Truncated AR (AR V7) From (Antonarakis,E.S. 2014)

Rationale for Combinational Therapy:

Abiraterone-resistance is majorly associated with reactivation of AR signaling. The data from various trials suggest that AR and enzyme 3β-hydroxysteroid dehydrogenase (3β-HSD converts DHEA to ASD which is then acted upon by 5α reductase to produce DHT) are inhibited by higher concentrations of abiraterone, hence dose escalation may be a viable strategy to target AR-related mechanisms of abiraterone resistance. This concept is currently under evaluation in two studies of men with metastatic CRPC (NCT01503229, NCT01637402)(University of Washington, 2015)(Friedlander, 2014)(Mostaghel, 2014a)

Multiple ongoing studies are evaluating the combination of abiraterone and enzalutamide or ARN-509(novel anti-androgen) in men with metastatic CRPC (NCT01650194, NCT01949337, NCT01792687)(Astellas Pharma Global Development, Inc., 2014)(Alliance for Clinical Trials in Oncology., 2015)(Aragon Pharmaceuticals, 2015) as

well as in men with localized disease prior to prostatectomy (NCT01946165)(M.D. Anderson Cancer Center., 2015). This is based on the concept that abiraterone-resistant tumors have persistent AR signaling, thus rationalizing the combination of abiraterone with potent AR inhibitors such as enzalutamide or ARN-509 (Mostaghel, 2014a)
The Systemic Therapy in Advancing or Metastatic Prostate Cancer: Evaluation of Drug Efficacy (STAMPEDE) study is another ongoing trial comparing ADT (androgen deprivation therapy) with and without abiraterone in high-risk patients with biochemical recurrence or newly diagnosed metastatic patients (NCT00268476)(Medical Research Council, 2014)(Mostaghel, 2014a). The efficacy of abiraterone and ADT in combination with salvage radiotherapy for biochemical recurrence following prostatectomy is being evaluated in another study (NCT01780220)(UNICANCER,)(Mostaghel, 2014a).
Neoadjuvant studies of multi-targeted AR blockade using LH-releasing hormone agonists combined with bicalutamide, dutasteride, and ketoconazole or LH-releasing hormone agonists combined with abiraterone have demonstrated higher pathologic response rates than previously observed in historic studies of ADT prior to prostatectomy (Mostaghel et al., 2014)(Taplin, et al., 2012) .

Abiraterone is a relatively safe and easily administered drug; hence it is an attractive first line therapy in asymptomatic or minimally symptomatic mCRPC patients. Sipuleucel T is a well-tolerated immunotherapy which has shown improvement in overall survival of mCRPC patients. Thus combination therapy with abiraterone and sipuleucel-T would probably be a very well tolerated regimen and is currently under clinical investigation (Mostaghel and Lin, 2014). Taxanes inhibit AR transcriptional activity by induction of transcriptional co-repressors as well as prevention of microtubule-mediated transit of AR to nucleus. Hence cross resistance to taxanes may develop due to resistance to hormonal AR pathway inhibitors. It becomes important to assess the efficacy of subsequent chemotherapy after treatment with abiraterone and enzalutamide. Retrospective analysis of docetaxel post abiraterone treatment showed decline of ≥50% PSA levels in only 26% patients compared to 45% in TAX327 study. Moreover no response was observed in abiraterone refractory patients with docetaxel treatment. This clearly showed that the

efficacy of docetaxel is greatly reduced with prior abiraterone exposure(Mezynski et al., 2012)(Mostaghel and Lin, 2014).

Androgen receptors are targeted directly, to overcome the resistance to abiraterone. Enzalutamide, a potent antagonist at androgen receptor, may be able to overcome the effects of increased transcription of full length androgen receptor and its splice variant(Tran et al., 2009). A phase II trial showed that enzalutamide improved survival in men with CRPC following progression after docetaxel, similar to the effects produced by abiraterone and cabazitaxel. Thus, individually, abiraterone and enzalutamide induce antagonistic changes in the androgen axis that could potentially be abrogated with combination therapy, supporting studies of combined abiraterone and MDV3100 (Stein, et al., 2012)

Metastatic prostate cancer most commonly metastasizes to bones. Major symptoms as well as complaints from the patients result from the bone disease. Such bone metastasis is osteoblastic giving them as sclerotic appearance as well as osteoclastic observed by rise in bone alkaline phosphatase (bone formation marker) and urinary N-telopeptide (marker for bone collagen breakdown). Clinical trials have shown the positive effect of two drugs in reversing this bone loss. Zolendronic acid (Bisphosphonate) and Denosumomab (a monoclonal antibody against RANK ligand) have proved to be very effective in inhibiting osteoclastic activation. Hence co-administration of these drugs with anti-androgens has proved to be very beneficial for improving bone disease symptoms (Saad et al., 2002)(Rosen et al., 2003)

Abiraterone combined with drugs targeting DHT synthesis such as dutasteride which is a 5α reductase, may also help to overcome abiraterone resistance(Figure 32) (Stein et al., 2012). Ketoconazole given in combination with dutasteride, effectively inhibits conversion of testosterone to the more active DHT [110]. Similarly a newer therapy like AKR1C3, drug targeting other enzymes involved in DHT synthesis, is under development (Mostaghel and Plymate, 2011).

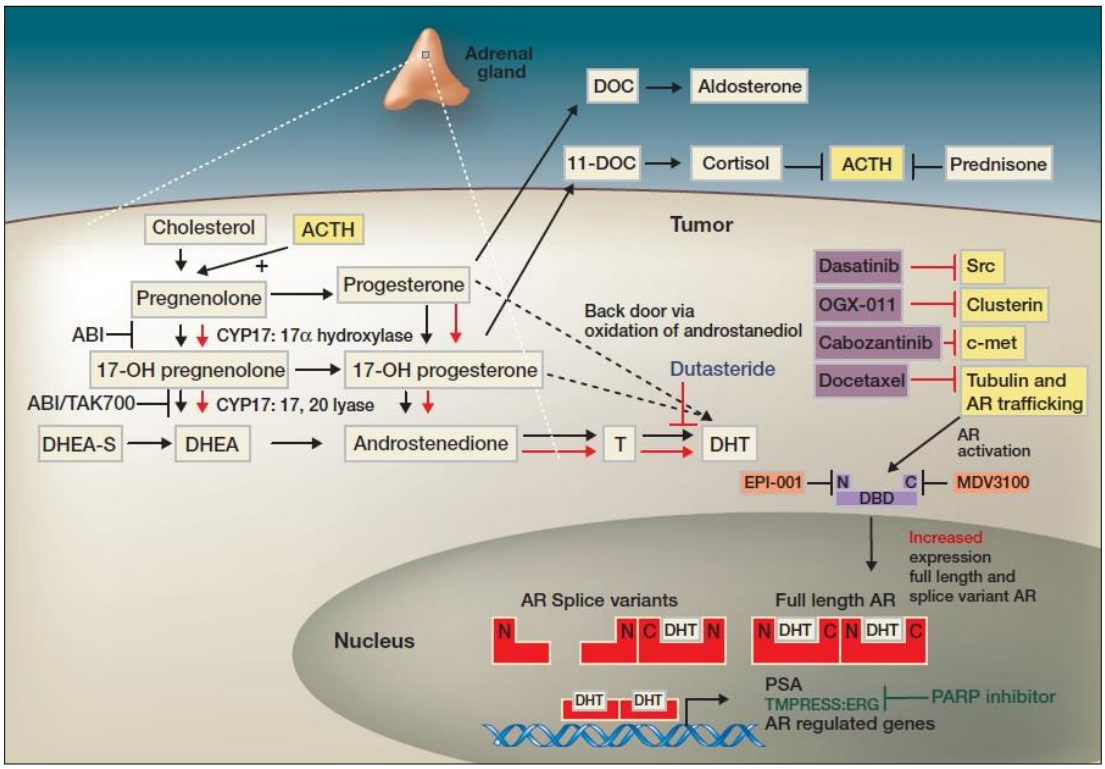


Figure 32: Therapies targeting the androgen-signaling axis. Abiraterone (ABI) is a potent and selective inhibitor of CYPc17, blocking synthesis oftestosterone and DHT. Other agents, such as MDV3100, target the androgen receptor directly. Resistance to abiraterone is proposed to occur throughupregulation of intratumoral CYP17 and other genes involved in synthesis of intratumoral androgens to restore DHT levels and through increased levels of androgen receptor and receptor splice variants. Abiraterone resistance pathways are depicted in red. AR, androgen receptor; DOC, deoxycorticosterone; 11-DOC, 11-deoxycortisol.. From (Stein et al., 2012)

Conclusion and future directions:

Understanding the molecular causes of a disease is very critical for finding successful treatment modalities. Inhibition of androgen receptor activation had been the key focus of prostate cancer treatment for many decades. Adrenal androgens, *denovo* intratumoral androgen synthesis and persistent AR activity after first-line hormonal therapy, has given rise to advanced disease – castration resistant prostate cancer. Resistance to first line therapy paved way for generation of molecular targeted therapy, thus making individualized rationale approach a necessity. Novel agents have now been discovered that target the androgen axis like inhibitors of androgen synthesis and androgen receptor antagonists.

Irreversible inhibition of CYP17A enzyme with abiraterone generated potent antitumor activity. Phase I/II/III studies showed that increased PSA response (decline in PSA ≥50%) with abiraterone therapy was further supported by radiological response, increase in overall survival, and improvement in symptoms as well as better quality of life. Major side effect with abiraterone was rise in ACTH and mineralocorticoid excess. This was counteracted by coadministration of low dose glucocorticoid (prednisone) or mineralocorticoid receptor antagonist.

Enzalutamide, a novel second generation AR antagonist, is another drug for patients with mCRPC post-docetaxel. It has much higher affinity for AR than previous anti androgens. It inhibits nuclear translocation along with DNA binding of AR without agonist activity. It is efficacious and safe drug, well tolerated by the patients and phase III clinical trial has demonstrated an improvement in overall survival of patients. It extends the progression free survival and is associated with enhanced response rates providing health related quality of life benefits. It does not require concomitant corticosteroid administration.

Clinical trial data for abiraterone and enzalutamide matures every day with correlative studies assessing the utility of circulating tumor cells(CTC). Meanwhile, identification of biomarkers to characterize patients most likely to respond will optimize the therapeutic

outcome of these agents. Unique radiolabelled isotopes, 18F-dihydrotesterone uptake, was assessed in Phase I/II trial of Enzalutamide whereas PET imaging was used in Phase III trials of MDV3100 and the latter result was generally concordant with PSA declines(Pal et al., 2009; Scher et al., 2010). Clinical responses to abiraterone are remarkable as seen in Phase I/II/III trials, but there is also development of resistance to abiraterone with reactivation of AR signaling, increased expression of CYP17A and induction of ligand- independent AR splice variant. Various mechanism for the resistance has been suggested, prominent being AR activation by concomitant glucocorticoid therapy. These results provide a strong rationale for combinational therapy consisting of abiraterone with potent AR inhibitor like enzalutamide in mCRPC patients. Other combinations with multi-targeted AR blockade have shown higher pathologic response rates (Mostaghel and Lin, 2014). Currently, various clinical studies are going on to evaluate the sequencing and combining of abiraterone, enzalutamide with immunotherapy, chemotherapy and other AR targeted agents at distinct stages of disease. Completion and analysis of these studies will lend us a better insight for determining the most rationale and efficacious treatment strategy for mCRPC.

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