UNIVERSITAT DE BARCELONA

Prostate Cancer: Mortality and Impact of Second-Generation Androgen-Receptor Inhibitors

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Prostate Cancer: Mortality and Impact of Second-Generation Androgen-Receptor Inhibitors

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A mis padres, ya que sin ellos
no hubiese llegado hasta aquí.

Index

1. Introduction

Prostate cancer is the second most frequently diagnosed cancer worldwide and the fifth leading cause of cancer death in men, with an estimated 1.1 million new cases diagnosed and 307.000 deaths in 2012 (1). In the European Union, the incidence of prostate cancer was 70 per 100.000 men in 2012 (2), with a mortality of 30.6 per 100.000 men/year and almost 90.000 deaths/year from prostate cancer which makes it the third most common cancer death in men in Europe (3). Importantly, there has been a large increase in prostate cancer incidence in the EU and worldwide over the past 20-30 years likely due in part to the widespread use of transurethral resection of the prostate and prostate-specific antigen (PSA) testing (4).

Prostate cancer is a heterogeneous disease ranging from indolent localized cases to aggressive undifferentiated cases with significant tendency to spread and become metastatic and incurable. Despite, the above mentioned incidence and mortality rates, the existence of a small proportion of patients who have indolent prostate cancers has led to the widely held belief that prostate cancer has low lethality and that most patients with prostate cancer will die with, rather than from their disease. The widespread use of PSA screening in asymptomatic patients in some countries such as in the United States of America (USA) might partially explain this belief. It is known that early PSA testing tends to overdiagnose a significant proportion of indolent tumours at earlier stages some of which may have remained latent and were not destined to cause symptoms or death (5). Consequently, overdiagnosis of indolent prostate cancers might have diluted the real mortality rates of more aggressive and advanced prostate cancers. Epidemiological studies analyzing real prostate cancer mortality and causes of death in countries were

early PSA screening is uncommon are therefore urgently needed to provide a real picture of the impact of prostate cancer on mortality.

Prostate cancer is usually suspected on the basis of an abnormal digital rectal examination and/or an incidental elevated PSA. Most cases of prostate cancer are diagnosed as localized disease (96%) and can be considered for radical treatment options such as prostatectomy and radical radiotherapy (6). Following radical treatment to localized disease, around 30% of patient will experience distant metastatic relapse (7). A small proportion of men with prostate cancer will present with metastatic disease at first presentation (4%), termed de novo metastatic prostate cancer (6); a subgroup of patients with worse outcome compared to patients relapsing after radical treatment. Primary androgen deprivation therapy (ADT) with luteinizing-hormone releasing hormone (LHRH) analogues or bilateral orchidectomy is the standard of care first-line treatment for metastatic prostate cancer patients.

Initial therapy with ADT results in a significant reduction of PSA levels in most patients (90-95%) and a reduction in tumour growth in around 75-85% of them (8, 9). However, following a median response to ADT of around 18 to 24 months, most patients will ultimately experience disease progression, developing castration-resistant prostate cancer (CRPC) (8, 9). Disease at this state was previously cataloged as androgen-independent or hormone-refractory. However, these terms are inaccurate and no longer in use since recent studies have shown that even at this stage, disease progression is still mainly driven by androgen receptor (AR) signaling. Consequently, in the last years several second-generation AR pathway inhibitors have been successfully tested in patients with metastatic CRPC confirming that prostate cancer progression remains dependent on

androgen stimulation. The potent inhibitor abiraterone acetate and the antiandrogen enzalutamide have both shown prolonged overall survival (OS) and improved quality of life in patients with metastatic CRPC previously treated or not with docetaxel chemotherapy (10-13). Furthermore, several other non-hormonal therapies such as the taxane chemotherapy agents docetaxel and cabazitaxel, radio-isotope alpha-emitter radium-223 and autologous cellular immunotherapy agent sipuleucel-T have also shown improved OS in prospective clinical trials and have been approved for the treatment of metastatic CRPC (14-18). The advent of these agents has revolutionized the management of patients with metastatic CRPC and significantly improved their survival thanks to their sequential use.

However, despite an initial response to these new drugs, most patients will ultimately experience drug-resistance leading to disease progression and a reduced survival. A better understanding of the underlying mechanisms of drug resistance is therefore crucial in order to improve the survival of prostate cancer patients. In addition, the approval of these several anticancer drugs has also raised the question of what is the ideal sequence for administering them. Importantly, the administration of additional lines of therapy in a given patient may allow sequential acquisition of mutations and cancer clonal evolution that may reduce the activity of subsequent treatment lines. Actually, the randomized clinical trials which led to the approval of both enzalutamide and abiraterone, did not allow the inclusion of patients who had previously been treated with the other drug, respectively. Therefore, although the individual efficacy of abiraterone and enzalutamide in prostate cancer is well established, the therapeutic benefit of targeting AR signaling axis by sequential administration of these agents is not clear. Actually, preliminary retrospective data has been published that suggests abiraterone has a reduced antitumour

activity when given after enzalutamide as compared to when given to enzalutamide-naïve patients, which indicates the existence of cross-resistance between these 2 agents (19, 20). Conversely, the antitumour activity of enzalutamide after abiraterone treatment in metastatic prostate cancer is still unknown but it may be hypothesized that cross-resistance might similarly occur.

The molecular mechanisms behind cross-resistance between second-generation AR inhibitors remain unclear. The prior experience gained with first-generation AR such as bicalutamide might provide us with some hypothesis to better understand them. Before the advent of second-generation agents, the use of bicalutamide as second-line hormone treatment in patients with metastatic prostate cancer progressing on ADT was very common. For those patients who later progressed whilst receiving bicalutamide, discontinuation of the drug had been reported to result in a paradoxical decrease of PSA levels in 15-30% of patients (21, 22) in what it is known as antiandrogen withdrawal syndrome (AAWS). Although the molecular mechanisms behind AAWS are not fully understood, the most accepted mechanism is AR gene mutations leading to alterations in the AR ligand-binding domain, which cause antiandrogens to act as partial agonists (23). While an AAWS has been reported for almost all first-generation antiandrogens such as bicalutamide or flutamide, there is no evidence of any withdrawal effect with second-generation antiandrogen enzalutamide. Unlike bicalutamide, no AR agonist effect was shown in enzalutamide preclinical studies (24). Hence, given the fact that enzalutamide is a second-generation pure AR antagonist, it is believed that enzalutamide may not have an AAWS. However, the existence or not of an AAWS with enzalutamide has never been investigated in the clinical setting. The hypothetical discovery of an enzalutamide AAWS

could provide some light into the potential molecular mechanisms of resistance and cross-resistance with second-generation AR inhibitors.

2. Epidemiology of Prostate Cancer

Prostate cancer has the highest incidence of all solid tumours in Europe, which is estimated to be 70 cases per 100.000 men/year (2), outnumbering lung and colorectal cancer. The worldwide prostate cancer burden is expected to grow to 1.7 million new cases and 499.000 new deaths by 2030 mainly due to the growth and aging of the global population (25). The highest estimated prostate cancer incidence rates occur in the highest resource areas of the world including the USA, Australia and New Zealand, and Europe (26). The incidence of prostate cancer also varies throughout Europe with a higher incidence in Western compared with Eastern and Southern Europe (26). The wide variation in international prostate cancer incidence rates is partly due to the significant differences worldwide in the access to early diagnosis of latent cancers through PSA screening test of asymptomatic cases and partly due to different environmental and risk factors. Regarding mortality rates, prostate cancer is the fifth most common cause of cancer death in men (1) behind lung, liver, stomach, and colorectal cancer. Despite the highest incidence rates occurring in developed countries, the highest estimated prostate cancer mortality rates tend to be seen elsewhere, mainly in the low- to medium-resource areas of South America, the Caribbean, and sub-Saharan Africa (26). In addition, since 1985, there has been a slight increase in most countries in the mortality from prostate cancer, even in countries where prostate cancer is uncommon (27).

The factors that cause prostate cancer remain largely unknown, although a few risk factors have been identified. Known factors that increase the risk of prostate cancer include age,

family history, and race. Prostate cancer affects elderly men more often than young men, with the peak incidence occurring in men >65 years of age. It occurs at an increasing rate with advancing age and it is rare before the age of 50 years. The median age of diagnosis of prostate cancer is 66 years, with the majority of diagnoses occurring in the 65-75 age group. Age-adjusted incidence rates per 100.000 men sharply decline after age 75 (28). During his lifetime, a 50-year-old man has a 42% chance of developing histological evidence of prostate cancer, a 9.5% risk of developing clinically important disease, and a 2.9% risk of death from prostate cancer (29). It is therefore a bigger health concern in developed countries where there is a greater proportion of elderly men as compared to developing countries. For instance, around 15% of male cancers are prostate cancer in developed countries compared to 4% in developing countries (30).

Family history remains the better characterized risk factor for developing prostate cancer. An estimated 9-10% of prostate cancers are due to hereditary predisposition (31) while the other 90% of the cases are considered sporadic. Some epidemiological studies have even shown that prostate cancer may have the highest familial cancer rate of any cancer: a single large study found a familial prostate cancer rate of 20.2%, compared to 13.6% for breast cancer and 12.8% for colorectal cancer (32). The risk of prostate cancer increases with the number of affected relatives. If a man has 1 first-degree relative affected with prostate cancer, the risk of developing the disease is at least doubled compared to that of the normal population. In men with 2 first-degree relatives with prostate cancer, the relative risk is increased 5-fold; and in those with 3 relatives, there is an 11-fold increase in risk of developing prostate cancer (33, 34). A true hereditary prostate cancer syndrome is defined as 3 or more affected relatives in the family, or at least 2 relatives who have developed early onset disease (before age 55) (34). Men with

a positive family history of prostate cancer usually present with the disease at an earlier age, around 6-7 years prior to sporadic cases, but do not differ in other clinical ways (34, 35). The exception to this are carriers of germ-line mutations in the BRCA2 gen or carriers of somatic alterations such as fusion in the TMPRSS2-ERG genes, who tend to have an increased risk of early-onset prostate cancer with aggressive behavior (36, 37).

Regarding ethnicity, black race is associated with a higher incidence of prostate cancer than other ethnic groups and is more likely to present with a higher Gleason grade (38). Black men also have a higher rate of high-grade prostatic intraepithelial neoplasia (PIN) and a more advanced pathologic stage at presentation compared to white men (39). However, despite this, the stage- and grade-adjusted mortality rates are similar between the 2 races (40). Japanese and Chinese ethnicities, meanwhile, have particularly low rates of prostate cancer (41). Data from the American National Cancer Institute Surveillance Epidemiology and End Results (SEER) database showed that African American men across all age groups have a higher incidence of advanced disease compared to age-matched men of other ethnic groups (42). This was confirmed by an epidemiological study which evaluated prostate glands on autopsies from 1.056 black and white men who died of causes other than prostate cancer and compared it to data from a radical prostatectomy database (43). While the autopsy data showed that subclinical prostate cancer in black and white men do not differ by race at early ages, radical prostatectomy data revealed that prostate cancer volume and Gleason grade were greater in black than in white men. Advanced or metastatic prostate cancer also occurred at a 4:1 ratio in black and white men. This supports the concept that prostate cancer grows more rapidly in black than in white men and that transformation from latent to aggressive prostate cancer occurs earlier in black than in white men (43).

Several environmental factors have also been linked to an increased prostate cancer risk, although their true impact on the causality of the disease remains unclear. The frequency of incidentally- and autopsy-detected cancers is approximately the same in different areas of the world (44) yet the incidence of clinical prostate cancer differs extensively between different geographical areas, as early mentioned. While the rates of prostate cancer are low in Japan, when Japanese people move to the USA, their risk increases significantly, approaching that of American men (45). These findings indicate that environmental factors affect the risk of progression from latent to clinical prostate cancer. Factors such as diet, sexual behavior, alcohol consumption, recurrent infective prostatitis, metabolic syndrome and occupational exposure have all been described as etiologically important risk factors of prostate cancer. The mechanism by which environment promotes prostate cancer development has been linked to chronic inflammation, suggesting that cell and tissue injury inflicted by dietary carcinogens, metabolic syndrome, and microbial pathogens, leads to a chronically inflamed prostatic milieu prone to induce neoplastic transformation (46). Persistent tissue damage, inflicted over many decades, may induce the corruption of genome integrity in normal prostate cells and trigger cancer initiation.

Among environmental factors, diet is one of the most studied factors for increased risk of prostate cancer. Dietary factors that may promote prostate cancer development include high intake of dietary fat and cooked meat, and low intake of fruits and vegetables (47). However, several studies have failed to support these findings and further data are needed to prove their association as risk factors. Finally, metabolic syndrome which includes obesity, hyperglycemia, dyslipidemia and hypertension has been associated with an increased risk of prostate cancer. A recent meta-analysis of studies that evaluated the

association between metabolic syndrome and prostate cancer showed that metabolic syndrome was associated with a 12% increase in prostate cancer risk. Among metabolic syndrome components, only hypertension and waist circumference >102 cm were associated with a significant greater risk of prostate cancer ($p<0.05$) (48).

Despite this increasingly profound knowledge on prostate cancer epidemiology, no medical intervention has so far been proved to reduce prostate cancer risk or mortality. Consequently, early detection of prostate cancer through regular digital rectal examination and PSA screening are the only interventions which could lead to an early diagnosis and potential increased survival.

3. PSA screening

The prostate-specific antigen (PSA) is a glycoprotein enzyme secreted by the epithelial cells of the prostate gland whose function is to enable spermatozoa to swim normally. PSA is physiologically present at small levels, usually below 4 ng/dL in the serum of men with healthy prostates. In men with adenocarcinoma of the prostate, PSA is almost invariably elevated and is used for risk stratification in localized diseased and as a prognostic tool in metastatic disease; greater levels of PSA being associated with more aggressive disease and a higher likelihood of advanced disease (49). During the late 1980s, PSA testing was shown to allow early diagnosis of the disease in asymptomatic men and was consequently adopted as a screening test in many countries. However, there is still no level 1 evidence that PSA screening increases survival and it remains one of the most controversial topics in prostate cancer literature. The goals of an opportunistic screening in oncology are the reduction of cancer-specific mortality (CSM), the improvement in OS and a maintained quality of life (50).

Population-based screening of men aged between 50 and 74 years using PSA testing, has been evaluated in 5 randomized trials (51-55). The larger study, called European Randomized Study of Screening for Prostate Cancer (ERSPC), was a double-blind randomized, multicenter trial initiated in the early 1990s aiming to compare mortality from prostate cancer in an intervention group (PSA testing every 2-4 years) compared to a control group with no intervention offered (51). The study randomized 162.388 healthy men aged 50 to 74 years. After 13 years of follow-up, 7.408 prostate cancer cases were diagnosed in the intervention group and 6.107 cases in the control group (56). The trial demonstrated a relative reduction in the risk of prostate cancer mortality of 21% (rate ratio 0.79, 95% confidence interval [CI] 0.69-0.91, p=0.001) in the intervention group (27% if adjusted for non-compliance). However, 781 men needed to be screened and 27 patients needed to be treated to prevent 1 death from prostate cancer (56). OS was not analyzed. The second larger study, the Prostate, Lung, Colon, Ovary (PLCO) Screening Trial was initiated around the same time and had a similar design (52). The study assigned 76.685 healthy men aged 55 to 74 years to receive either annual PSA screening or usual care as the control. At 13 years of follow-up, 4.250 participants had been diagnosed with prostate cancer in the intervention arm compared with 3.815 in the control arm (57). However, the cumulative mortality rates from prostate cancer were 3.7 deaths per 10.000 person-years in the intervention compared to 3.4 in the control arms, resulting in a non-statistically significant difference between the 2 arms (relative risk 1.09, 95% CI 0.87-1.36) (57). Likewise, OS was not analyzed. The 2 largest randomized clinical trials provided therefore conflicting results for only one of them was positive.

In view of these controversial results, a Cochrane review and meta-analysis of the 5 available randomized clinical trials (including 341.342 participants) was conducted in order to better determine if PSA screening reduces prostate CSM or all-cause mortality (58). PSA screening was significantly associated with an increased diagnosis of prostate cancer (risk ratio 1.30, 95% CI 1.02-1.65). Moreover, PSA screening was also associated with more localized disease (risk ratio 1.79, 95% CI 1.19-2.70) and less advanced prostate cancer (risk ratio 0.80, 95% CI 0.73-0.87). However, the meta-analysis indicated no statistically significant difference in prostate CSM between men randomized to the screening and control groups (risk ratio 1.00, 95% CI 0.86-1.17). Moreover, the meta-analysis of the 4 studies that inverstigated OS did not find any significant differences between men randomized to screening or control (risk ratio 1.00, 95% CI 0.96-1.03) (58). Conversely, PSA screening was associated with common minor harms such as bleeding, bruising and short-term anxiety and with common major harms including overdiagnosis and overtreatment. The risk of overdiagnosing indolent tumours at earlier stages due to PSA screening is clinically very relevant as it is associated to the overtreatment of many cases which may have remained latent and might never have caused symptoms or death.

All these considerations have led to a strong advice against systematic population-based screening in Europe and the USA which is reflected in the most prestigious international guidelines on prostate cancer (50, 59). A risk-adapted strategy for early detection might still be offered on an individual basis to well-informed men with an elevated risk of having prostate cancer (men ≥50 years with a positive family history) (50). In any case, men should always be informed of the risk of overdiagnosis and treatment-related harms when asking about whether or not to undertake screening for prostate cancer. Any potential reduction in prostate CSM may take up to 10 years to accrue; therefore, men

who have a life expectancy less than 10 to 15 years should be informed that screening for prostate cancer is unlikely to be beneficial (58). Finally, despite the controversial role of PSA as a screening tool, its use is still very important for treatment response monitorization in advanced disease following AR pathway blockade.

4. Targeting the Androgen Receptor in Prostate Cancer

4.1 The Androgen Receptor Axis and Androgen Deprivation Therapy

The androgen receptor (AR) is a nuclear receptor transcription factor highly expressed in normal prostate epithelial cells whose main function is to regulate genes required for the synthesis of proteins involved in the production of seminal fluid (60). In the absence of its main ligands, testosterone and dihydrotestosterone (DHT), the AR is mainly located in the cytoplasm. Following the androgen binding to the ligand-binding domain, the AR changes its conformation which triggers its translocation into the nucleus (61). In the nucleus, AR dimerizes and binds to the DNA where it acts as a transcription factor regulating the expression of genes involved in the normal functioning of the prostatic gland.

In normal human male physiology, androgen stimulation of the AR is fundamental to normal prostate cells growth. Serum androgens are mainly synthesized in the testes and the adrenal gland (62). The Leydig cells of the testes produce testosterone, which acts as an agonist stimulator of the AR. The synthesis of testicular testosterone is regulated by the luteinizing hormone (LH) which is secreted by the pituitary gland, under the upstream stimulation by the LHRH, released by the hypothalamus. Testosterone is converted by the enzyme 5α-reductase in peripheral tissues, including benign and malignant prostatic tissues, to the more potent DHT which is also an agonist of the AR. Although the

multistep pathway of androgen synthesis from cholesterol to testosterone is present only in its entirety in the testes, the adrenal cortex is also capable of synthesizing and secreting androgens, including dehydroepiandrosterone (DHEA) and androstenedione. These so-called mild androgens do not usually biologically stimulate the AR but can be converted by enzymatic processes to testosterone and DHT.

Because prostate cancer cells derive from androgen-sensitive normal prostate cells, they usually retain its sensitivity to androgen stimulation at least during the first phases of the carcinogenic process leading to prostate cancer. Likewise, this sensitivity is mainly driven by the stimulation of prostate cancer cells AR by androgens. The discovery that depletion of circulating gonadal testosterone with surgical or medical castration with ADT resulted in the regression of metastatic prostate cancers was first realized in the 1940s by Charles Huggins and Clarence Hodges (63). That discovery was so transcendental that medical or surgical castration has since then been the cornerstone treatment for metastatic prostate cancer and it even earned Charles Huggins the Nobel Prize of Medicine in 1966. In the early 1980s the first synthetic LHRH agonists were developed. Chronic administration of these LHRH agonists was found to produce an inhibitory effects over the pituitary gland, causing a suppression of circulating levels of follicle-stimulating hormone (FSH) and LH. This medical castration produced a massive reduction in serum testosterone levels equal to those caused by surgical castration (64). Several synthetic LHRH agonists have been so far developed for clinical use; leuprolide and goserelin being the 2 more frequently used. These LHRH agonists have been tested in a large number of randomized trials comparing the different approaches of ADT, such as bilateral orchiectomy and LHRH agonists. These studies showed that both approaches are equally effective in causing a 90-95% reduction in both testosterone and PSA levels and a reduction in tumour growth

in 75-85% of patients (8, 9). In view of these studies, LHRH agonists have become the standard of care ADT for treating newly diagnosed metastatic prostate cancer patients.

4.2 Castration Resistance

ADT with LHRH analogues is the gold standard first-line treatment for patients with metastatic prostate cancer. However, despite an excellent initial PSA and clinical response, most patients will ultimately experience disease progression after a median time of response of 18 to 24 months (8, 9). Selection pressure induced by maintained androgen deprivation treatment leads to the emergence of a tumor phenotype characterized by disease progression despite castrate levels of testosterone ($\leq$50 ng/dL) which is nowadays known as castration resistance.

Several molecular mechanisms that could lead to castration resistance have been described. It has been shown that ADT induces increases in the expression of AR, either due to AR gene amplification or mRNA or protein overexpression. Overexpression of AR sensitizes the receptor to low levels of androgens and prostate cancer cells can therefore proliferate despite castrated levels of testosterone (65). Other alterations include AR mutations leading to AR that have lost the binding domain and are constitutively active without ligands or other mutations causing AR to bind to additional ligands that would normally not stimulate the wild-type receptor, such as corticosteroids (66, 67). In addition to AR-related abnormalities, other relevant molecular mechanisms leading to CRPC transformation have been described, such as the up-regulation of adrenal and intratumoral androgen biosynthesis. Several studies have shown that as much as 10% of baseline circulating testosterone remains in castrated men, due to peripheral conversion of adrenal steroids to testosterone (68). These studies have shown that despite treatment

with medical castration, prostate cancers continue to have sufficient levels of androgens to drive tumor growth not only thanks to peripheral conversion of adrenal steroids but also due to intratumoral androgens biosynthesis. It has been shown that castration-resistant prostate cancer cells overexpress the enzymes required for androgen biosynthesis leading to relatively high levels of intratumoral testosterone concentrations, as compared to levels measured in the blood (69). The increasing knowledge of the molecular mechanisms behind castration-resistant have led to the understanding that disease progression to ADT is still mainly driven by the AR. Consequently, patients progressing to LHRH analogues are no longer considered androgen-independent or hormone-refractory but castration-resistant. This change of paradigm contributed to promote the drug development of first generation antiandrogens as well as more modern AR axis inhibitors such as abiraterone acetate or enzalutamide with the aim of overcoming resistance to ADT.

4.3 First-generation antiandrogens and the antiandrogen withdrawal syndrome

The discovery of the AR in the late 1960s was followed by a search of agents that could block the AR binding domain. Several antiandrogens were synthetically created, all sharing the same mechanism of action of competitively binding to the AR and thus displacing the capacity of androgens to bind to the AR. First-generation antiandrogens were classified according to their chemical structure as steroidal (cyproterone acetate, megestrol acetate and medroxyprogesterone acetate) and non-steroidal (nilutamide, flutamide and bicalutamide). While steroidal anti-androgens have progestational properties leading to a decrease in testosterone levels, non-steroidal antiandrogens do not lower testosterone, which remain normal or, conversely, slightly increased. This latter characteristic was associated with a more favorable safety profile as compared to LHRH

analogues as they did not reduce libido or potency. Bicalutamide, the most studied and common first-generation antiandrogen was initially used as monotherapy instead of ADT in castration-sensitive metastatic patients. However, although bicalutamide was better tolerated, it was shown to be inferior than ADT when given in monotherapy, in terms of OS and progression-free survival (PFS) (70, 71).

The next step was to combine ADT, aiming to reduce the levels of testosterone, plus an antiandrogen, aiming to block at the AR level the effect of residual androgens produced by the adrenal glands, a concept known as combined or maximum androgen blockade (MAB). A great number of randomized clinical trials were undertaken comparing MAB with monotherapy ADT. Although a meta-analyses showed that MAB appeared to provide a small survival advantage of less than 5% versus monotherapy (72) in metastatic patients, the use of MAB is still controversial as several of these studies were underpowered and had methodological flaws (50). However, despite the lack of level 1 evidence, MAB was the most commonly used second-line hormone treatment in patients with metastatic prostate cancer progressing on ADT. Other secondary hormonal manipulations, such as the addition of oral estrogen stilbesterol or corticosteroid prednisolone or dexamethasone were also frequently utilized in patients progressing on ADT despite the lack of randomized clinical trials proving their benefit. Before the advent of the second-generation AR axis inhibitors such as enzalutamide or abiraterone acetate, these secondary hormonal manipulation were so common that they were considered as a mandatory requisite in order for a patient to be considered castration-resistant and to meet the criteria to enter clinical trials or to receive docetaxel chemotherapy.

For those patients who progressed whilst receiving a first-generation antiandrogen, discontinuation of the antiandrogen had been reported to produce paradoxical reductions in PSA levels in 15-30% of patients (21, 22, 73) in what it is known as the antiandrogen withdrawal syndrome (AAWS). Similarly, the withdrawal of the antiandrogen was also considered a valid secondary hormonal manipulation. The most accepted definition of AAWS is a decline in PSA level of ≥50% from baseline after antiandrogen cessation, with a confirmed decrease 3-6 weeks later (73). The AAWS in prostate cancer was described for the first time in 1993 after discontinuation of the non-steroidal antiandrogen flutamide (74, 75). Subsequently, similar withdrawal responses were reported in patients treated with other non-steroidal antiandrogens, such as bicalutamide (21, 22, 73, 76) and nilutamide (22, 73, 77, 78), as well as with steroidal antiandrogens, such as cyproterone acetate (79) and megestrol acetate (80). While symptomatic benefit and objective radiographic responses have been reported in some cases of AAWS, no impact on survival has ever been shown. However, and despite a low level of evidence, the withdrawal of the antiandrogen was a very common therapeutic maneuver in the first-generation antiandrogen era. While some long-term maintained withdrawal responses have been reported, for most patients experiencing an AAWS, further anticancer treatment was finally needed after a median PSA response duration of 3.5-5.0 months because of disease or PSA progression (22).

With the advent of second-generation AR axis inhibitors, the practice of secondary hormonal manipulation, including the antiandrogen withdrawal, was abandoned and is no longer considered as a mandatory requisite to fulfil the criteria of CRPC (50). Nevertheless, the AAWS was a critical discovery because it increased our understanding of the biology of the AR and the mechanisms leading to castration resistance. The

molecular mechanisms underlying AAWS have not been fully determined partly because of the lack of suitable experimental models. The most accepted mechanism is AR gene mutations leading to alterations in the AR ligand-binding domain, which cause antiandrogens to act as partial agonists (23). To date, several preclinical studies using prostate cancer cell models have shown that specific mutations in the AR gene are responsible for the switch of bicalutamide (81) and flutamide (82) from AR antagonist to partial agonist. It has been demonstrated in these preclinical models that sustained long-term therapy with antiandrogen can result in the induction of AR mutations, of which, if located on the ligand-binding domain, could cause antiandrogens to act as partial agonists. Consequently, the newly gained agonist effect would stimulate prostate cancer progression and would explain the reduction in PSA upon withdrawal of the agonistic antiandrogen. Moreover, the empirical clinical evidence that most patients who develop an AAWS had been receiving the antiandrogen therapy for a prolonged period of time, underpins the results seen in the preclinical models and the agonist transformation hypothesis.

4.4 Abiraterone acetate

Abiraterone acetate is a potent, selective, and irreversible inhibitor of cytochrome P450c17 (CYP17), a critical enzyme involved in androgen biosynthesis. CYP17 is a microsomal enzyme that has both 17α-hydroxylase and c17-20-lyase activities, and catalyzes 2 independently regulated reactions key to androgen and estrogen biosynthesis mainly in the adrenal gland but also in the testes (Figure 1). As mentioned earlier, extratesticular sources of testosterone represent an important alternative source of androgen stimulation in castrated patients with prostate cancer mainly due to peripheral conversion of adrenal steroids to testosterone. First-line treatment by ADT leaves the

testosterone derived from adrenal gland production intact. By blocking the role of CYP17, abiraterone triggers a significant reduction on this supplementary source of testosterone by the adrenal glands, but also within the testes and the prostatic tumor cells which also express CYP17.

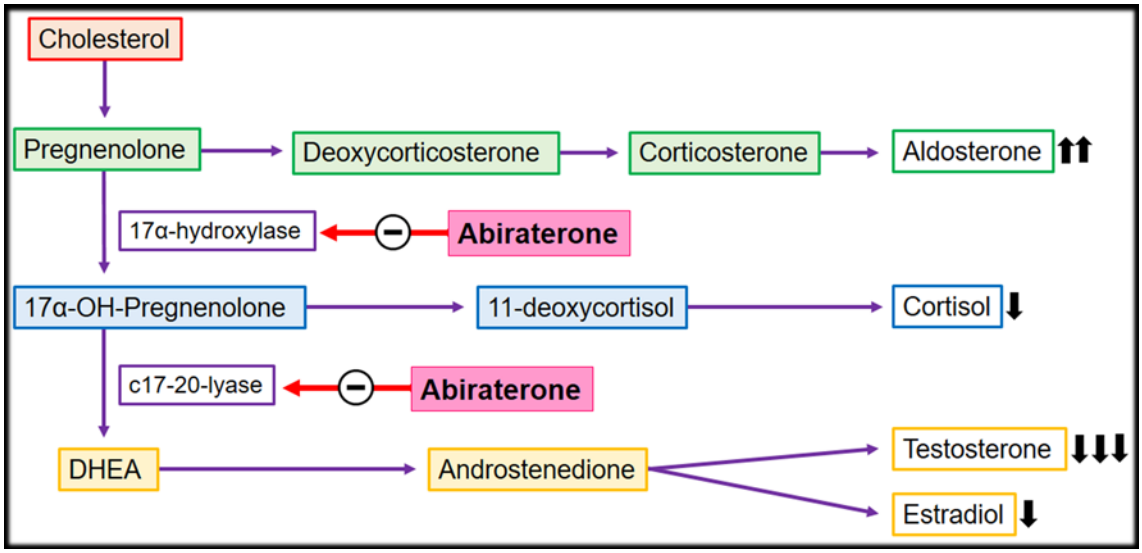

Figure 1: Steroid synthesis pathway, mechanism of action of abiraterone acetate and repercussion on final steroids

DHEA= dehydroepiandrosterone; 17α-OH-pregnenolone= 17α-hydroxypregnenolone.

As a consequence of the blockade of CYP17, there is a relative accumulation of precursor enzymes involved in the corticosteroid and mineralocorticoid synthesis. This leads to an increase of corticosterone and aldosterone which causes a secondary mineralocorticoid excess (Figure 1). Early clinical trials of abiraterone showed that his effect can be largely abrogated by the use of low-dose prednisone or prednisolone (5 mg twice daily). Consequently, concurrent low dose prednisone is now considered a standard of care component of abiraterone therapy.

Abiraterone acetate was first tested in the COU-AA-301 trial in patients with metastatic CRPC who have previously received docetaxel chemotherapy (10). This was a phase III, multinational, randomized, double-blind study of abiraterone-prednisone compared to prednisone plus placebo. Abiraterone was given orally at the standard dose of 1.000 mg daily continuously and prednisone at 5 mg orally twice daily. The study enrolled 1.195 patients of which 89% had bone metastases and 10% had liver metastases. Importantly, OS was significantly longer in the abiraterone acetate group than in the placebo–prednisone group (14.8 months versus 10.9 months; hazard ratio [HR] 0.65, 95% CI 0.54-0.77, $p<0.001$) resulting in a 35.4% reduction in the risk of death. The effect of abiraterone in OS was consistent across all subgroups including in elderly patients and in patients with visceral metastases. Abiraterone also resulted in remarkable PSA responses (29% versus 6%, $p<0.001$) and soft-tissue objective response rate (ORR) (14% versus 3%, $p<0.001$). It also significantly prolonged the time to first skeletal-related event (SRE) defined as pathological fracture, spinal cord compression, palliative radiation or surgery to bone (9.9 versus 4.9 months, $p\leq0.05$).

Given the good results of abiraterone acetate in the postdocetaxel setting, another phase III trial, the COU-AA-302 trial, was designed to assess the role of abiraterone in chemotherapy-naïve patients (12). In this international, randomized, double-blind study, 1.088 patients with chemotherapy-naïve metastatic CRPC were randomly assigned to receive abiraterone acetate plus prednisone or placebo plus prednisone. Abiraterone significantly prolonged median OS as compared to prednisone plus placebo (34.7 months versus 30.3 months, HR 0.81, 95% CI 0.70-0.93, $p=0.0033$) (83). The rates of PSA response (62% versus 24%, $p<0.001$) and radiographic ORR (36% versus 16%, $p<0.001$)

to therapy were also significantly higher in the abiraterone group than in the prednisone-alone group, respectively. Table 1 summarizes the main efficacy results of these 2 important randomized trials.

Description	n	Median age (range)	PSA response rates	Measurable disease response	Median PFS (months)	Median OS (months, 95% CI)
Phase III double-blind placebo-controlled trial in mCRPC after chemotherapy (COU-AA-301 trial) (10)	1.195	69 (39-95)	≥50%: 29% with A vs 6% with P (*p*<0.001)	PR + CR: 14% with A vs 2.8% with P (*p*<0.001)	5.6 with A vs 3.6 with P (HR 0.67, 95% CI 0.59-0.78, p<0.001)[a]	14.8 with A vs 10.9 with P (HR 0.65, 95% CI 0.54-0.77, *p*<0.001).
Phase III double-blind placebo-controlled trial in chemotherapy-naïve mCRPC (COU-AA-302 trial) (12)	1.088	71 (44-95)	≥50%: 62% with A vs 24% with P (*p*<0.001)	PR + CR: 36% with A vs 16% with P (*p*<0.001)	NR with A vs 8.3 with P (HR 0.43, 95% CI 0.35-0.52, *p*<0.001)[a]	34.7 with A vs 30.3 with P (HR 0.81, 95% CI 0.70-0.93, *p*<0.001)

Table 1: Randomized phase III clinical trials with abiraterone acetate in metastatic castration-resistant prostate cancer

mCRPC= metastatic castration-resistant prostate cancer; n= number of patients; PSA= prostate-specific antigen; PFS= progression-free survival; OS= overall survival; CI= confidence interval; NR= not reached; A= abiraterone-prednisone; P= placebo-prednisone; PR= partial response; CR= complete response; SD= stable disease; vs= versus; HR=hazard ratio.

a: radiographic progression-free survival

Both clinical trials showed a favorable safety and tolerability profile of abiraterone acetate. Treatment-related adverse events (AEs) were predominantly grade 1 or 2, and were mainly mechanism-based and secondary to mineralocorticoid excess. These included mild peripheral edema (28%-31%), hypopotassemia (17%) and hypertension

(10%-22%), and were largely mitigated by the use of low-dose prednisone (10, 12). The most common AEs in both trials were fatigue (39%-44%), bone pain (30%-32%), arthralgia (27%-28%) and nausea (22%-30%). Hematologic toxicity was infrequent and mainly at the expense of mild anemia (23%) with only <1% of grade 3-4 neutropenia or thrombocytopenia and no cases of febrile neutropenia. There was a low rate of drug discontinuation (19%) or dose reduction (3.5%) in both studies and these occurred at a similar frequency in the placebo groups. In both trials, abiraterone acetate was associated with hepatotoxicity in terms of elevation in aminotransferase levels in a minority of patients. In the COU-AA-301 study however, aminotransferase levels abnormalities occurred with a similar frequency in the abiraterone and placebo groups, including changes of any grade (10% and 8%, respectively) and grade 3 or 4 changes (3.5% and 3.0%). In the COU-AA-302 study, hepatotoxicity was more frequent in the abiraterone arm but rarely reached a grade 3-4 level: any grade increased alanine aminotransferase (ALT) (12% versus 5%), grade 3-4 increased ALT (5% versus <1%), any grade increased aspartate aminotransferase (AST) (11% versus 5%) and grade 3-4 increased AST (3% versus <1%). Nevertheless, abiraterone-related aminotransferase levels elevations are usually reversible and easily solved with brief treatment interruptions. Importantly, no patient in either clinical trial died from a hepatotoxicity.

These studies validated the hypothesis that the biosynthesis of androgens by the adrenal gland contributes to progression of CRPC since abiraterone acetate can produce tumor responses in patients who no longer benefit from standard ADT. Consequently, following the publication of the COU-AA-301 and COU-AA-302 trials, abiraterone acetate plus prednisone was approved by the Food and Drug Administration (FDA) and the European

Medicines Agency (EMA) for the treatment of metastatic CRPC patients previously treated or not with docetaxel.

4.5 Enzalutamide

Enzalutamide is a non-steroidal second-generation antiandrogen that competitively inhibits androgen binding to AR thus inhibiting androgen-related AR activation and prostate cancer cell stimulation. Unlike first-generation antiandrogens, enzalutamide also acts on other different steps of the AR signaling pathway: it also inhibits nuclear translocation of the AR, DNA binding to the AR, and coactivator recruitment further blocking AR protumoral functions (Figure 2). Moreover, enzalutamide binds to the AR with 5 to 8-fold greater affinity than bicalutamide and has not been shown to have any partial agonist effect on preclinical CRPC models unlike bicalutamide or flutamide (24).

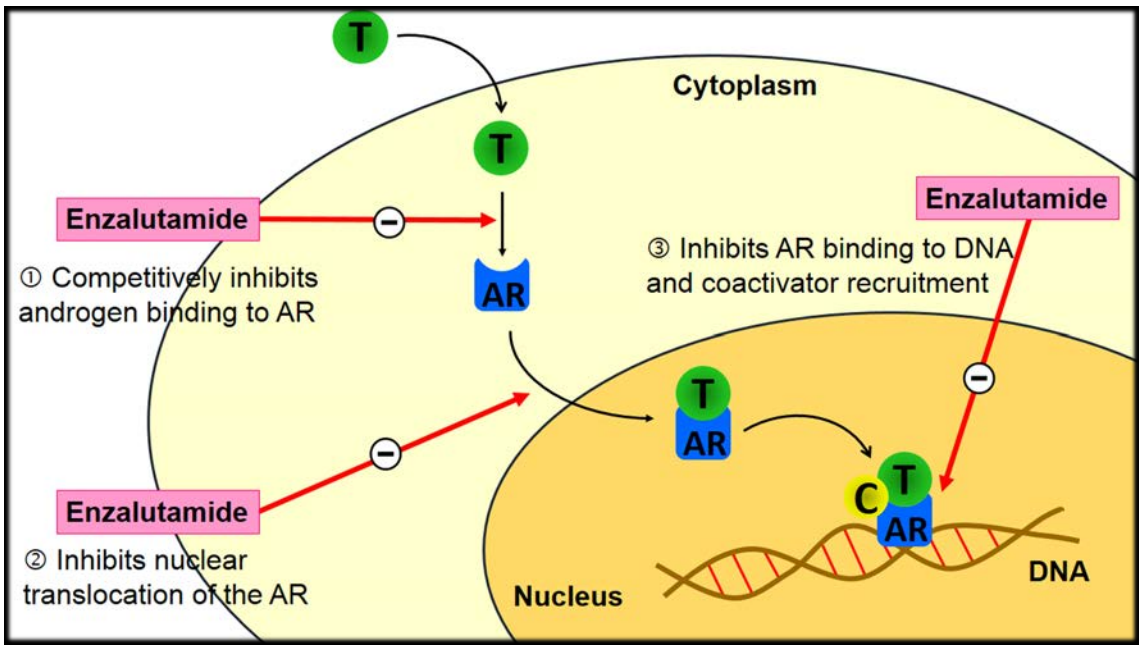

Figure 2: Mechanisms of action of enzalutamide in the androgen receptor axis (84)

AR= androgen receptor; T= testosterone; DNA= deoxyribonucleic acid; C= coactivator.

The AFFIRM trial was the first clinical study to test the role of enzalutamide in metastatic CRPC patients (11). It was an international, phase III, randomized, double-blind, study of enzalutamide compared to placebo in patients with prostate cancer who had previously been treated with 1 or 2 chemotherapy regimens, at least 1 of which contained docetaxel. Enzalutamide was given orally at the standard dose of 160 mg daily continuously. The study enrolled 1.199 patients of which 91.6% had bone metastases and 23% had visceral metastases in lung or liver. Importantly, enzalutamide significantly improved median OS compared to placebo (18.4 months, 95% CI 17.3-not reached versus 13.6 months) resulting in a reduction of 37% in the risk of death (HR 0.63, $p<0.001$). The survival benefit with enzalutamide was consistent across all subgroups, including the poor-risk categories such as Eastern Cooperative Group Performance Status (ECOG PS) 2, moderate or severe pain, visceral metastases and more than 20 bone lesions. Enzalutamide also resulted in remarkable PSA responses (54% versus 2%, $p<0.001$) and soft-tissue ORR (29% versus 4%, $p<0.001$). It also significantly prolonged the time to first SRE defined as need for radiotherapy or surgery to bone, pathologic bone fracture, spinal cord compression, or change of anticancer therapy to treat bone pain (16.7 versus 13.3 months, HR 0.69, $p<0.001$) independently of the use of bisphosphonates at baseline (85).

In view of the excellent results of enzalutamide in the postdocetaxel setting, another phase III trial, the PREVAIL study, was designed to assess the role of enzalutamide in chemotherapy-naïve patients (13). It was a multinational, double-blind, randomized, phase III trial of enzalutamide compared to placebo in men with metastatic CRPC who have progressed to ADT but have not received chemotherapy. A total of 1.717 patients were enrolled in the study with the majority of patients having bone metastases (83.3%),

half of them lymph node involvement (50.7%) and only 11.8% visceral metastases in lung or liver. Importantly, enzalutamide significantly prolonged OS compared to placebo (32.4 months in the enzalutamide group versus 30.2 months in the placebo group) thus reducing in a 29% the risk of death (HR 0.71, p<0.001). This benefit was also seen in all subgroups including elderly people (≥75 years), visceral metastases and low hemoglobin levels. The superiority of enzalutamide over placebo was shown for all secondary endpoints including PSA response (78% versus 3%, p<0.001), radiographic ORR (59% versus 5%, p<0.001) and time to first SRE (31.1 versus 31.3 months, HR 0.72, p<0.001). Table 2 summarizes the main efficacy results of these 2 important randomized trials.

Description	**n**	**Median age (range)**	**PSA response rates**	**Measurable disease response**	**Median PFS (months, 95% CI)**	**Median OS (months, 95% CI)**
Phase III double-blind placebo-controlled trial in mCRPC after chemotherapy (AFFIRM trial) (11)	1.199	69 (41-92)	≥50%: 54% with E vs 2% with P (*p*<0.001)	PR + CR: 29% with E vs 4% with P (*p*<0.001)	8.3 with E (8.2-9.4) vs 2.9 with P (2.8-3.4, HR 0.40, *p*<0.001)[a]	18.4 with E (17.3-NR) vs 13.6 with P (11.3-15.8, HR 0.63, *p*<0.001).
Phase III double-blind placebo-controlled trial in chemotherapy-naïve mCRPC (PREVAIL trial) (13)	1.717	72 (43-93)	≥50%: 78% with E vs 3% with P (*p*<0.001)	CR: 20% with E vs 1% with P PR: 39% with E vs 4% with P (*p*<0.001)	NR with E vs 3.9 with P (HR 0.19, *p*<0.001)[a]	NR with E vs 31.0 with P (HR 0.73, *p*<0.001)

Table 2: Randomized phase III clinical trials with enzalutamide in metastatic castration-resistant prostate cancer

mCRPC= metastatic castration-resistant prostate cancer; n= number of patients; PSA= prostate-specific antigen; PFS= progression-free survival; OS= overall survival; CI= confidence interval; NR= not reached; E= enzalutamide; P= placebo; PR= partial response; CR= complete response; SD= stable disease; vs= versus; HR=hazard ratio.

a: radiographic progression-free survival

In terms of safety and tolerability, enzalutamide is generally a well-tolerated drug and has a favorable toxicity profile. The rates of serious AEs were similar between enzalutamide and placebo both in the AFFIRM and PREVAIL phase III trials (34% versus 39%; and 32 versus 27%, respectively) (11, 13). Enzalutamide-related AEs are usually mild and grade ≥3 AEs are uncommon (28% for all AEs and between 1% and 6% for each individual AE). The most common AE with enzalutamide is fatigue (34-36%) and it rarely attains a grade ≥3 level (2-6%). Other frequent AEs are osteoarticular pain (14-27%), constipation (22%), diarrhea (16-21%), hot flushes (18-20%) and hypertension (6.6-13%). Importantly, no relevant hematologic, biochemical or electrolyte AEs were reported in any of the 2 phase III trials. AEs leading to treatment discontinuation or death were rare (6-8% and 3-4%, respectively). Worth mentioning is a potentially severe but very infrequent AE with enzalutamide: the occurrence of seizures. A seizure was reported in 5 patients with enzalutamide in the AFFIRM trial (0.6%) and in 1 patient in the PREVAIL trial (0.1%). In both clinical trials patients with a prior history of seizure or a condition that could confer a predisposition to seizure were excluded. However, the majority of patients who experienced a seizure during these trials had predisposing factors which could have lowered the threshold for convulsion. Consequently, enzalutamide cannot be administered to patients with central nervous system metastases or history of seizures.

These 2 pivotal phase III trials of enzalutamide were relevant because they showed that disease progression during the castration-resistant setting is still mainly driven by the AR. CRPC patients are therefore not hormone-resistant since an antiandrogen such as enzalutamide can improve survival despite the acquired resistance to ADT. Consequently,

following the publication of the AFFIRM and PREVAIL trials, enzalutamide was approved by the FDA and the EMA for the treatment of metastatic CRPC patients previously treated or not with docetaxel.

5. Hypotheses and objectives

5.1 Hypotheses:

-The first hypothesis of this research study is that mortality of prostate cancer remains significant despite the incorporation of new-generation drugs such as enzalutamide or abiraterone, especially in a setting where the uptake of PSA screening is low such as in the United Kingdom (UK). This challenges the traditional belief that prostate cancer is not an important cause of death in men.

-The second hypothesis is that the activity of enzalutamide in advanced and heavily pre-treated castration-resistant prostate cancer patients previously treated with abiraterone is significantly reduced as compared to when enzalutamide is given at earlier stage of disease in abiraterone-naïve patients. This reduced activity of enzalutamide in abiraterone pre-treated patients indicates cross-resistance between these 2 hormonal drugs which target the AR signaling pathway.

-The third and last hypothesis is that maintained treatment with enzalutamide may lead to the development of a partial AR-agonist effect illustrated by the appearance of an AAWS with enzalutamide discontinuation. The underlying molecular mechanisms of AAWS could provide some insight into the mechanisms of drug resistance to enzalutamide.

5.2 Objectives:

1. To investigate mortality and causes of death in men with prostate cancer diagnosed in London (UK) where routine screening testing for PSA amongst asymptomatic men is low. To examine the relationship between cause of death and patient characteristics at

diagnosis including age, cancer stage, and treatment received during the first 6 months following diagnosis. To calculate for each of the above factors the overall proportion of total deaths at each level that were due to prostate cancer, and the cumulative incidence functions for deaths.

2. To analyze the antitumour activity of enzalutamide in terms of PSA response, radiologic response and survival in metastatic castration-resistant prostate cancer patients previously treated with abiraterone and to compare it with published data on the efficacy of enzalutamide when given to abiraterone-naïve patients.

3. To examine PSA levels following enzalutamide discontinuation in metastatic castration-resistant prostate cancer patients, in order to assess whether an antiandrogen withdrawal syndrome exists with enzalutamide and to correlate post-withdrawal PSA levels with survival. To correlate patients' clinical and treatment factors associated with a potential antiandrogen withdrawal syndrome with enzalutamide.

6. Methods and Patients

6.1 Prostate Cancer Mortality Study:

Data on 53.081 men diagnosed with prostate cancer between January 1st 1997 and December 31st 2006 were extracted from the Thames Cancer Registry (TCR) database. The TCR is a population-based epidemiological registry, covering around 12 million people resident in an area of South East England comprising London, Kent, Surrey and Sussex. Since 1997, cause of death has been recorded routinely in coded format using the International Classification of Diseases codes (ICD-9 and ICD-10). Subjects were followed up to the end of December 2007, with a median follow up of 3.5 years. Only

patients with available clinical data (date of diagnosis, treatment received, cause of death) were included, in order to be able to assess survival and the relationship between cause of death and patient clinical characteristics. Following the removal of patients with incomplete or incongruous data, a total of 50.066 men were left for analysis.

The underlying cause of death was taken from the death certificate. Cause of death certificate is completed in the following manner with the instruction that 'Underlying Cause of Death' should appear in the lowest completed line of Part I and instruction on the death certificate as below:

- Part 1a: Disease or condition directly leading to death.
- Part 1b: Other disease or condition, if any, leading to 1a.
- Part 1c: Other disease or condition, if any, leading to 1b.
- Part 2: Records any significant condition or disease that contributed to the death but which is not part of the sequence leading directly to death.

The underlying cause of death was taken as that recorded in Part 1c of the death certificate if present, or if not then from Part 1b if present, or finally from Part Ia. If more than 1 cause was recorded in the relevant part, then the first of these was used (unless this was pneumonia, in which case the second cause was used). Specific recorded causes were classified as follows: prostate cancer, other urological cancers, lung cancer, colorectal cancer, other digestive cancers, other/unspecified cancers, ischaemic heart disease, other cardiovascular disease, pneumonia, and all other causes.

Data were collected on date of death, which was used together with date of diagnosis to calculate survival for each individual patient. Causes of death were tabulated in relation to age at diagnosis, stage of prostate cancer at the time of diagnosis, and type of treatment within 6 months of diagnosis. All treatment modalities received within 6 months of diagnosis were recorded and it was therefore possible for a given men to have more than 1 treatment recorded. The stages used for prostate cancer are as follows in relation to classical TNM staging (86).

- Stage 1: Local disease confined to the prostate (correlates with TNM: T1-3 N0)
- Stage 2: Tumour extends into local tissues/organs (correlates with TNM: T4 N0)
- Stage 3: Loco-regional lymph node involvement (correlates with TNM: any T, N1)
- Stage 4: Distant metastases (correlates with TNM: M1)

For each of the above factors, we calculated the overall proportion of total deaths at each level that were due to prostate cancer, and also the cumulative incidence functions for deaths from a number of causes, using the competing risks methodology of Fine and Gray (87). The latter are displayed as stacked graphs showing the cumulative deaths by time since diagnosis.

6.2 Enzalutamide Study:

6.2.1 *Eligibility*:

Patients with metastatic CRPC treated with enzalutamide in the UK between 2012 and 2013 were retrospectively identified. Clinical data were collected from the electronic patient records. Criteria for inclusion were histological diagnosis of prostate adenocarcinoma, ongoing ADT or bilateral orchidectomy, ECOG PS between 0-2,

adequate haematological, hepatic and kidney function and the absence of brain metastases or a history of seizures. Concomitant treatment with zoledronic acid or denosumab was allowed. Two different sub-studies were conducted in this same population with slight differences in the subgroups analyzed in each sub-study:

- Enzalutamide Cross-Resistance study: patients previously treated with both abiraterone and docetaxel and subsequently treated with enzalutamide were included in the Enzalutamide Cross-resistance study. Between June 2012 and February 2013 a total of 39 patients were identified.

- Enzalutamide Withdrawal study: Patients with evidence of disease progression on enzalutamide were selected for the Withdrawal study. Only patients with at least 1 available PSA post-enzalutamide discontinuation were included. Patients who received palliative radiotherapy immediately following enzalutamide cessation were excluded as were patients on low dose steroids, as these could cause the PSA to decrease. Between June 2012 and October 2013, 30 consecutive patients were initially identified. A subsequent updated analysis increased the patient sample to 49 patients.

6.2.2 *Treatment plan and evaluations*:

Enzalutamide was administered orally at a dose of 160 mg once daily. Dose reductions of 25% or 50% (to 120 or 80 mg once daily respectively) were allowed in case of toxicity. Baseline evaluations prior to treatment initiation included medical history and physical exam, baseline imaging assessments with computed tomography (CT) and bone scans, and baseline PSA levels. Clinical assessments with PSA levels and blood tests with full blood count and hepatic and renal function were performed every 4 weeks. Radiological

assessments were approximately every 3 to 6 months while on treatment. Following enzalutamide discontinuation, clinical assessment and PSA levels were performed for those patients included in the Enzalutamide Withdrawal study every 2 weeks until further anticancer treatment was started. CT and bone scans were performed 4 weeks after enzalutamide cessation when possible.

6.2.3 *Efficacy Endpoints*:

Descriptive analyses were performed to determine PFS and OS, PSA response and factors associated with response and survival. PFS was defined as time from enzalutamide initiation to disease progression in bone or soft tissue, symptoms or death. The interval between enzalutamide initiation and death from any cause was the definition for OS. Time on treatment was defined as the interval between date of first and last dose of enzalutamide. PSA response was defined as a PSA decline by ≥50% from baseline and PSA progression as PSA rise by 25% from the nadir, both confirmed with a second PSA value at least 3 weeks later. Following enzalutamide discontinuation, an enzalutamide withdrawal syndrome was defined as a PSA decline by ≥50% from the last on-treatment PSA, with a confirmed decrease 3 or more weeks later. A 3 weeks threshold was chosen, and not 6 weeks, because at 6 weeks most patients would have started further anticancer therapy and would not be evaluable. Radiological response and progression were evaluated according to Prostate Cancer Working Group criteria 2 (PCWG2) for bone scans and to Response Evaluation Criteria in Solid Tumours 1.1 (RECIST 1.1) for measurable disease on CT scans (22, 23). Clinical progression was a composite endpoint that included increased disease-related pain (assessed according to the 11-point numeric rating scale, with 0 representing the absence of pain and 10 representing the worst imaginable pain) (24), occurrence of SREs, indication for palliative radiotherapy or

cancer-related worsening of ECOG PS. Adverse events were graded using Common Terminology Criteria for Adverse Events (CTCAE) v4.0.

6.2.4 *Statistical analysis*:

Statistical analysis was performed with SPSS Statistics v20 (IBM), using as the cut-off date the 2nd of May 2013 and the 22nd of October 2013 for the Cross-resistance and Withdrawal sub-studies, respectively. Duration of treatment, OS and PFS were calculated using Kaplan-Meier estimates.

- Enzalutamide Cross-Resistance study: The association between PSA response on enzalutamide and OS, and of previous response to abiraterone and PFS on enzalutamide were explored using Cox regression models. The association of rates of ≥50% PSA response on previous abiraterone with rates of ≥50% PSA responses on enzalutamide was explored using binary logistic regression models.

- Enzalutamide Withdrawal study: The association between post-withdrawal PSA and OS was explored using Cox regression models. The prognostic association between clinical and treatment factors and a potential AAWS was studied using univariate logistic regression analyses.

7. Results (Articles)

7.1 Causes of death in men with prostate cancer: an analysis of 50.000 men from the Thames Cancer Registry (88)

Causes of death in men with prostate cancer: an analysis of 50 000 men from the Thames Cancer Registry

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S.C. and D.R. contributed equally to this work.

Objective

- To investigate causes of death in a UK cohort of patients with prostate cancer.

Patients and Methods

- We examined causes of death in a UK cohort of 50 066 men with prostate cancer diagnosed between 1997 and 2006 reported to the Thames Cancer Registry (TCR) and followed-up to the end of 2007.
- The underlying cause of death was taken from the death certificate.
- Uptake of PSA screening was low in the UK during the period studied.
- We examined the relationship between cause of death and patient characteristics at diagnosis including age, cancer stage, and treatment (≤6 months of diagnosis).

Results

- In all, 20 181 deaths occurred during the period; 49.8% recorded as being due to prostate cancer, 17·8% to cardiovascular disease, 11·6% to other cancers, and 20·7% to other causes.
- Irrespective of age, cancer stage, or treatment ≤6 months of diagnosis, prostate cancer was an important cause of death ranging from 31·6% to 74·3% of all deaths in different subgroups.

Conclusion

- For men with prostate cancer diagnosed in a setting where uptake of PSA screening is low, our findings challenge the belief that prostate cancer is not an important cause of death.

Keywords

prostate cancer, mortality, causes of death, PSA screening

Introduction

Cancer of the prostate is the commonest non-cutaneous cancer in men in the UK [1]. It accounts for nearly a quarter of all new male cancer diagnoses with a total of 40 975 men diagnosed in 2010 and is the second most common cause of male cancer death in the UK with a total of 10 721 deaths in the same year [1]. There has been a large increase in prostate cancer incidence in the UK and many other countries over the past 20–30 years [2]. The increased incidence in the UK is thought to be mainly due to increased rates of detection initially due to increased rates of TURP and subsequently the introduction of PSA testing in the early 1990s [3]. Despite this large increase in prostate cancer incidence, survival has improved and mortality rates have remained relatively constant. This may be the result of increased detection of earlier stage cancers, some of which may have remained latent and of questionable clinical significance.

In the USA there is a high level of PSA testing in asymptomatic men. A study from the National Cancer Institute showed that 33·6% of men aged 50–64 years had undergone a PSA test in the previous year [4]. This figure rose to 51·3% in those aged ≥65 years. In the UK so called 'opportunistic' PSA testing has a much lower penetrance of ≈6%, with only 2% of tests being performed in asymptomatic men [5,6]. It is thought that the high level of PSA testing in the USA has led to over diagnosis and subsequently unnecessary treatment in men whose prostate cancer was not destined to cause symptoms or death [7]. Welch and Albertsen [7] calculated that between 1986 and

Table 1 Recorded cause of death in men diagnosed with prostate cancer.

Cause of death	ICD-9 codes	ICD-10 codes	Deaths, n (%)
Prostate cancer	185	C61	10 053 (49.8)
Other cancers	140–239, excluding above	C00–C96 excluding above	2 355 (11.6)
CVD	390–405 +410–459	100–115 +120–199	3 587 (17.8)
Other causes	All other death codes	All other death codes	4 186 (20.7)
Total	–	–	20 181 (100)

2005, PSA testing had resulted in an additional one million men being diagnosed and treated for prostate cancer, with most of this excess being due to over diagnosis. Consequently, there is a widely held belief that men with prostate cancer will die with, rather than from, their disease.

In the present study, we have investigated causes of death in a UK cohort of men with prostate cancer, where routine testing for PSA amongst asymptomatic men is low. We also examined the effects of age, stage and treatment on cause of death in these men.

Patients and Methods

Data on 53 081 men diagnosed with prostate cancer between 1 January 1997 and 31 December 2006 were extracted from the Thames Cancer Registry (TCR) database. TCR is a population-based registry, covering ≈12 million people resident in an area of South East England comprising London, Kent, Surrey and Sussex. Since 1997 'cause of death' has been recorded routinely in coded format using the International Classification of Diseases codes (ICD-9 and ICD-10; http://www.cdc.gov/nchs/icd/). Patients were followed up to the end of December 2007, with a median follow-up of 3.5 years.

We excluded 2601 men registered only from a death certificate (4.9% of the total) as these men did not have a diagnosis date to calculate survival and also lacked information on treatment received. In all, 284 men (1·4% of the deaths) with unknown cause of death were also excluded. Men in which the initial treatment included cystoprostatectomy were excluded when there was a diagnosis of bladder cancer ≤6 months of the diagnosis of prostate cancer (122 men). This was because it was felt that the bladder cancer was likely to be the dominant pathology for these men. Eight additional men were excluded as they had a recorded date of death preceding the recorded date of diagnosis. This left a total of 50 066 men for analysis.

The underlying 'cause of death' was taken from the death certificate. In the UK the death certificate is completed by a registered medical practitioner who has been in attendance during the deceased patient's last illness. Cause of death certificate is completed in the following manner with the instruction that 'Underlying Cause of Death' should appear in the lowest completed line of Part I and instruction on the death certificate as below:

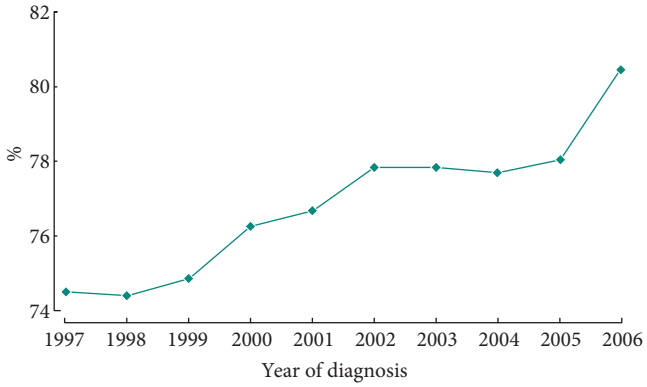
Part 1a: Disease or condition directly leading to death.
Part 1b: Other disease or condition, if any, leading to 1a.
Part 1c: Other disease or condition, if any, leading to 1b.
Part 2: Records any significant condition or disease that contributed to the death but which is not part of the sequence leading directly to death.

The underlying cause of death was taken as that recorded in Part 1c of the death certificate if present, or if not then from Part 1b if present, or finally from Part 1a. If more than one cause was recorded in the relevant part, then the first of these was used (unless this was pneumonia, in which case the second cause was used). This method was used in order to follow as closely as possible the recommendations for classifying underlying cause of death published by the Office for National Statistics [8]. Specific recorded causes were classified as follows: prostate cancer, other urological cancers, lung cancer, colorectal cancer, other digestive cancers, other/unspecified cancers, ischaemic heart disease, other cardiovascular disease (CVD), pneumonia, and all other causes. The corresponding ICD-9 and ICD-10 codes are shown in Table 1.

Data were collected on date of death, which was used together with date of diagnosis to calculate survival for each individual patient. Those for whom date of death was recorded as the same as date of diagnosis (352 men) were included in the analysis by assigning half a day's survival to each. Causes of death were tabulated in relation to age at diagnosis, stage of prostate cancer at the time of diagnosis, and type of treatment ≤6 months of diagnosis. All treatment methods received ≤6 months of diagnosis were recorded and it was therefore possible for men to have more than one treatment recorded. The radical surgery group was defined as all men recorded as having been treated with a prostatectomy ≤6 months of diagnosis. The stages used by TCR for prostate cancer are as follows in relation to classical TNM staging.

Fig. 1 Prostate cancer incidence by year of diagnosis.

Fig. 2 Percentages of prostate cancer diagnoses with known stage classified as localised disease (stage 1) by year.



TCR stages of disease:

TCR Stage 1: Local disease confined to the prostate (correlates with TNM: T1–3)
TCR Stage 2: Tumour extends into local tissues/organs (correlates with TNM: T4)
TCR Stage 3: Loco-regional lymph node involvement (correlates with TNM: N1)
TCR Stage 4: Distant metastases (correlates with TNM: M1)

For each of the above factors, we calculated the overall proportion of total deaths at each level that were due to prostate cancer, and also the cumulative incidence functions for deaths from a number of causes, using the competing risks methodology of Fine and Gray [9]. The latter are displayed as stacked graphs showing the cumulative deaths by time since diagnosis.

Results

Figure 1 shows the incidence of prostate cancer by year of diagnosis, based on registration data from TCR. There was a steep increase up to 2001. Thereafter, incidence rates have remained relatively constant. Figure 2 shows the proportion of prostate cancer diagnoses where stage was known that were classified as localised. During this period there was also an increase in the percentage of men with localised prostate cancer.

By the end of the study period there had been 20 181 (40.3%) deaths out of the 50 066 men with prostate cancer. Table 1 shows the recorded causes of death in these men. Almost half of the deaths (10 053 men; 49.8%) were due to prostate cancer. The next most common cause of death was CVD with 3587 deaths (17.8%). Other common recorded causes of death were other cancers (11.6%) and other causes (20.7%).

Table 2 shows the age distribution for the 50 066 men. The 65–74 and ≥75 years age groups constitute the largest groups with 39.0% in each group. The stage distribution for patients is shown in Table 3. Most men, 27 717 (55.4%), had stage 1 or localised disease. Stages 2 and 3 are relatively uncommon for prostate cancer, so these groups were combined due to low numbers, with 1168 men (2.3%). The under-representation of stage 2 and 3 in our cohort may represent under staging as relatively few patients may have had radiological or surgical staging. There were 7112 men (14.2%) with stage 4 or metastatic disease. Information on stage was unknown for 14 069 (28.1%) men. Table 4 shows treatment received ≤6 months of diagnosis. Radical surgery was performed on 8.4% of the men, with 16.0% and 36.8% receiving radiotherapy (RT) and hormonal therapy, respectively. In all, 23 517 men (46.3% of the total) had no recorded treatment. The total number of treatments (53 767) exceeds the total number of men (50 066), as some men had more than one treatment recorded in the first 6 months after diagnosis. By far the most common combined therapy was hormone therapy, and radiotherapy, which was recorded for 3988 men (8.0%).

Figure 3 shows graphically the proportions of all patients dying from each of the major causes (prostate cancer, CVD, other cancers, and other causes) during the course of the study. This analysis allows for competing causes of death and provides estimates of the proportions of men dying from each cause of death as a cumulative function of survival time. In the first column we see the effect of age on the analysis. With increasing age the percentage of prostate cancer deaths as a proportion of all deaths falls (65.6% <65 years: to 46.4% ≥75 years) as other causes, most notably CVD, become more important. The proportion of deaths rises with increasing age but in all age groups prostate cancer remains the predominant single cause of death.

The second column in Figure 3 examines the effect of stage on cause of death. Stage exerts a major effect on the percentage of deaths from prostate cancer itself, rising from

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Table 2 Causes of death by age.

	Age, years			Total
	<65	65–74	≥75	
N (%)	10 992 (21.9)	19 563 (39.1)	19 511 (39.0)	50 066
Number of deaths (all causes)	1 953	5 832	12 396	20 181
Number of prostate cancer deaths (% of all deaths)	1 282 (65.6)	3 014 (51.7)	5 757 (46.4)	10 053 (49.8)

Table 3 Causes of death by stage.

	Stage:				Total
	1	2–3	4	Unknown	
N (%)	27 717 (55.4)	1168 (2.3)	7112 (14.2)	14 069 (28.1)	50 066
Number of deaths (all causes)	8 663	378	6019	5 121	20 181
Number of prostate cancer deaths (% of all deaths)	3 094 (35.7)	192 (50.8)	4474 (74.3)	2 293 (44.8)	10 053 (49.8)

Table 4 Causes of death by treatment*.

	Treatment:				Total
	Radical surgery	Radiotherapy	Hormonal therapy	Unknown	
N (%)	4197 (8.4)	7987 (16.0)	18 426 (36.8)	23 157 (46.3)	50 066†
Number of deaths (all causes)	380	2397	9 144	9 165	20 181
Number of prostate cancer deaths (% of all deaths)	120 (31.6)	1455 (60.7)	5 124 (56.0)	3 962 (43.2)	10 053 (49.8)

**Causes of death by treatment of 50 066 men with prostate cancer diagnosed between 1997 and 2006 reported to the TCR and followed up to the end of 2007.*
†Total men with prostate cancer is 50 066 and total number of treatments is 53 767. This is because some men had more than one treatment recorded in the first 6 months after diagnosis.

35.7% of all deaths in localised (stage 1) disease to 74.3% for metastatic (stage 4) disease.

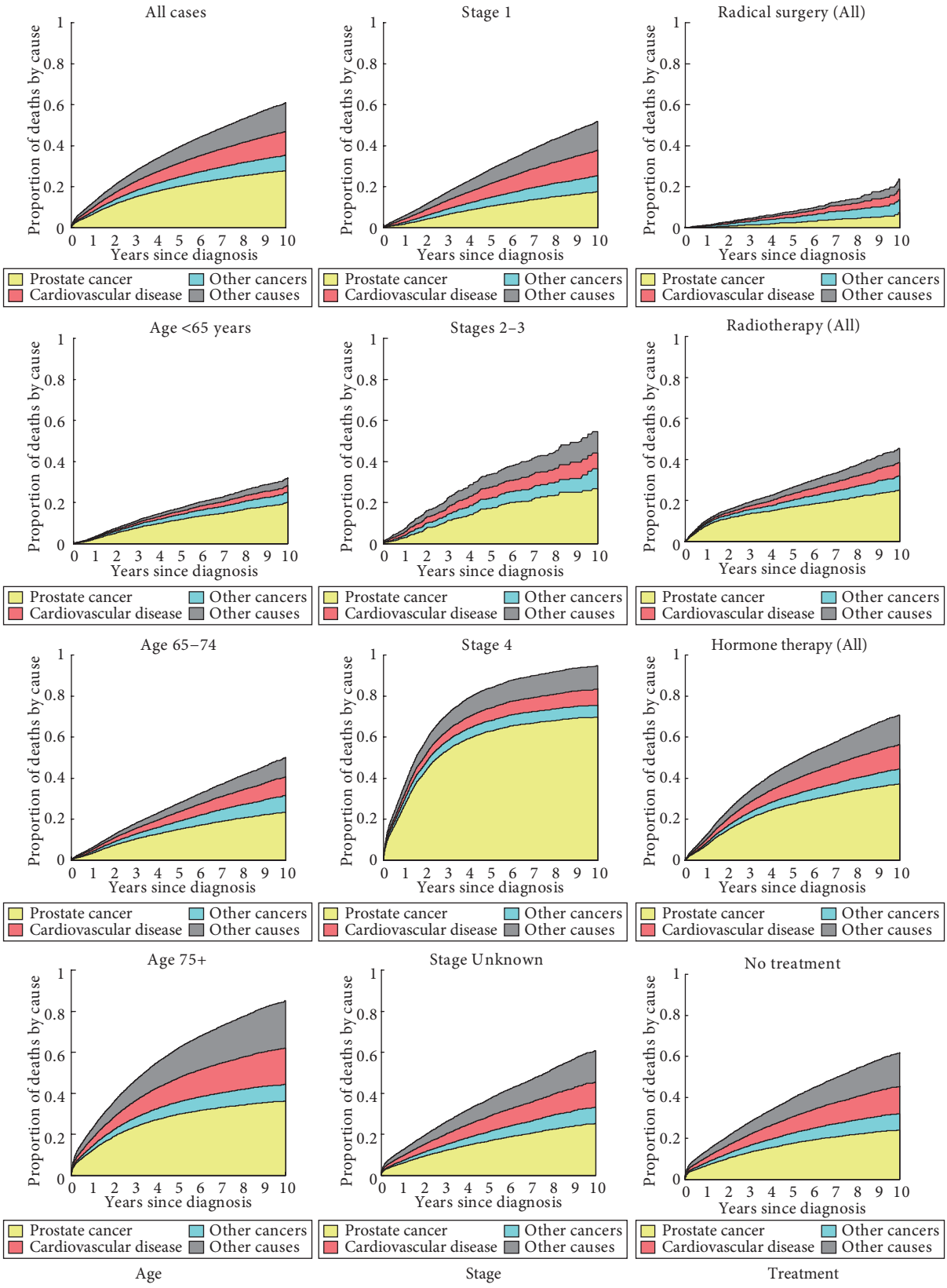
The third column in Figure 3 examines the proportion of deaths by treatment ≤6 months of diagnosis. For men treated with radical surgery, 4197 men, the number of deaths is lower (380 men) but death from prostate cancer is still a significant proportion of all deaths at 31.6% in this group. The surgical group are younger (58% aged <65 years; 4.5% aged ≥75 years) and 72% are recorded as having stage 1 disease. The radiotherapy group, 7987 men, is older with most (53·2%) aged 65–74 years and 19·8% aged ≥75 years. This group is also more heterogeneous, with 15.1% of men having recorded metastatic disease and possibly receiving palliative radiotherapy. The radiotherapy group has a high number of total deaths (2397) and the proportion of all deaths, which are due to prostate cancer is 60.7%. The hormonal therapy group, 18 426 men, is the oldest group, with 43.4% of men aged ≥75 years and only 16.2% of men aged <65 years. This group also contains the largest proportion of men with recorded metastatic disease (23·9%). The hormonal therapy group has a large number of total deaths (9144) with a high proportion of all deaths due to prostate cancer (56.0%).

The final treatment group shown is those who had no record of treatment ≤6 months of diagnosis. This group is the largest group with 23 157 men. Stage 1 is the commonest stage for these men (53.3% of men). The proportion of all deaths in this group from prostate cancer is 43.2%.

Discussion

The present study shows several important findings. The most striking is the high proportion of deaths from prostate cancer. This occurred in 20% of the men studied despite a median follow up of only 3.5 years. Prostate cancer was an important cause of death in all the subgroups we studied irrespective of age, stage or treatment. Stage 1 disease was the commonest stage seen but even in this group, prostate cancer remained an important cause of death accounting for 35·7% of all deaths. Important findings related to treatment are that only 11% of men with stage 1 disease were recorded as having radical surgery ≤6 months of diagnosis. In addition, 46.3% of all men had no record of treatment ≤6 months of diagnosis.

Fig. 3 Stacked cumulative incidence functions for various causes of death by age, stage and treatment.



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The age groups 65–74 and ≥75 years were the two commonest age groups reflecting the fact that prostate cancer is most prevalent in older men. However, in all age groups prostate cancer remained the predominant cause of death. It is important to remember this when making treatment decisions in men of all ages with prostate cancer, by doing so we will avoid underestimating the lethal nature of prostate cancer in men of all ages with clinically detected disease and the potential to do harm by under treatment.

Other studies have analysed the cause of death in patients with localised prostate cancer in populations where PSA screening is more prevalent [10,11]. Unsurprisingly the cancer-specific mortality (CSM) rate has been shown to be much lower than the present study. Abdollah et al. [11] investigated the CSM and other-cause mortality rates in 404 604 North American men with localised prostate cancer only. The 10-year CSM rate was 6.1% and prostate cancer was the cause of death in only 16.6% of all deaths. In the present study, 49.8% of the deaths were recorded as being due to prostate cancer. It is likely that the differences seen are due to several factors. We think that the most significant of these are that the present analysis also included men with locally advanced and metastatic disease from a population where PSA screening is low.

Compared with data from the Surveillance, Epidemiology and End Results (SEER) taken from USA cancer registries there are some notable differences. The SEER stage distribution allocates only T1 and T2 disease as localised disease whereas the definition of localised disease used by TCR includes T3 disease. The latest SEER data on 5-year survival by stage at diagnosis for 2003–2009 shows 81% of cases are classified as localised, 12% regional, 4% metastatic, and 3% unknown [12]. The 5-year relative survival was 100% for those men with localised or regional disease and overall 5-year relative survival was 99·4%. Stage 1 disease in the present cohort encompasses all local disease (T1–3) and is likely to be dominated by symptomatic disease with higher local stage and grade. This contrasts with the SEER data where the group with localised disease is dominated by asymptomatic men detected by PSA testing who often have low volume and grade disease. We think the high proportion of deaths in stage 1 in the present cohort reflects the presence of men with locally advanced disease for whom prostate cancer is the most significant pathology. It is also important to note that surgery, radiotherapy, systemic treatment and supportive care have all improved during the period of the present study and therefore the percentage of men dying from prostate cancer in a contemporary cohort may be lower.

The steep increase in incidence up to 2001 is probably due to improved awareness of prostate cancer and better diagnostics. This includes increased use of DRE, improved imaging with TRUS to facilitate prostate biopsies as well as increased use of PSA testing after its introduction in the early 1990s. Thereafter, incidence rates have remained relatively constant, which is consistent with data available from the whole of the UK during this period [1]. There was also an increase in the percentage of cases of localised prostate cancer, consistent with rising awareness of the disease and better diagnostic techniques as outlined above. This stage distribution, with a predominance of localised disease, is comparable with that seen from other UK groups [13]. However, despite these changes, prostate cancer remains an important cause of death in all the subgroups defined by stage.

A large number of men were recorded as having no treatment and also in this group prostate cancer was a common cause of death. This group contained men with low-grade, low-volume disease who underwent active surveillance and those with significant co-morbidities who are only treated when they become symptomatic – the 'watchful waiting' group. This group will also contain men who were managed with active surveillance or watchful waiting during the first 6 months after diagnosis, but who then proceeded to treatment with surgery, radiotherapy, and hormonal therapy or combinations of these. Finally, it also contains men who did not have initial treatment recorded and thus were misclassified for type of treatment. However, even if the no treatment group is heterogeneous for prognosis, a substantial proportion of these men are those deemed to be suitable for conservative management, and still prostate cancer death constitutes a large proportion of all deaths.

To our knowledge this is the largest study of this kind and benefits from the central collection and analysis of data. However, assessment of cause of death is important for the present study and is a potential weakness. Studies of the use of death certificates to determine cause of death in prostate cancer have shown variable results and it remains uncertain as to how accurate this method is [14–16]. The most recent and relevant study is by Godtman et al. [16] using an independent cause of death committee that showed a 96% overall agreement with the initial cause of death using death certificates. Another potential weakness is the relatively short duration of follow-up for some men in the present study. Prostate cancer is a heterogeneous disease with variable outcomes, but even men who present with metastatic disease are likely to benefit from androgen-deprivation therapy and their survival is likely to be significantly longer than 1 year [17]. It seems likely therefore that with increasing follow-up of the present cohort of men the number and proportion of men dying from prostate cancer will increase further. Notably there are large numbers of men where stage and/or treatment

were not known. Whilst this may affect the exact estimates and interpretation relating to a specific stage and/or treatment it does not affect the overall estimates of the proportion of men dying from prostate cancer.

In conclusion, for men with prostate cancer diagnosed in a setting where uptake of PSA screening is low, our findings challenge the notion that prostate cancer is not an important cause of death. This situation is not unique to the UK and our findings are important for many countries with a high risk of prostate cancer but low PSA testing. As other causes of death become more common in men with prostate cancer with longer follow-up time, and thus with increasing age, these other causes do not eradicate prostate cancer death as is sometimes thought. Rather, they are added to the total risk of death and prostate cancer death remains the most important health problem in a greater than a 10-year perspective. The present data highlight one of many challenges to the healthcare system in the management of prostate cancer and reflect some of the complexity of the disease.

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Conflict of Interest

Lars Holmberg is also funded by Cancer Research UK.

Ethics Approval

The authors followed the World Medical Association's Declaration of Helsinki. The present study did not require Ethics approval, as there are no identifiable patients.

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Abbreviations: **CSM**, cancer-specific mortality; **CVD**, cardiovascular disease; **ICD**, International Classification of Diseases (codes); **SEER**, Surveillance, Epidemiology and End Results; **TCR**, Thames Cancer Registry (database).

7.2 Antitumour activity of enzalutamide in patients with metastatic castration-resistant prostate cancer pre-treated with docetaxel and abiraterone (89)

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Antitumour activity of enzalutamide (MDV3100) in patients with metastatic castration-resistant prostate cancer (CRPC) pre-treated with docetaxel and abiraterone

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KEYWORDS
Abiraterone
Enzalutamide
Castration resistant prostate cancer
Hormone therapy
Androgen receptor targeting

Abstract ***Background:*** The new generation anti-androgen enzalutamide and the potent CYP17 inhibitor abiraterone have both demonstrated survival benefits in patients with metastatic castration-resistant prostate cancer (CRPC) progressing after docetaxel. Preliminary data on the antitumour activity of abiraterone after enzalutamide have suggested limited activity. The antitumour activity and safety of enzalutamide after abiraterone in metastatic CRPC patients is still unknown.
Patients and Methods: We retrospectively identified patients treated with docetaxel and abiraterone prior to enzalutamide to investigate the activity and safety of enzalutamide in a more advanced setting. Prostate specific antigen (PSA), radiological and clinical assessments were analysed.
Results: 39 patients with metastatic CRPC were identified for this analysis (median age 70 years, range: 54–85 years). Overall 16 patients (41%) had a confirmed PSA decline of at least 30%. Confirmed PSA declines of $\geqslant$50% and $\geqslant$90% were achieved in 5/39 (12.8%) and 1/39 (2.5%) respectively. Of the 15 patients who responded to abiraterone, two (13.3%) also had a confirmed $\geqslant$50% PSA decline on subsequent enzalutamide. Among the 22 abiraterone-refractory patients, two (9%) achieved a confirmed $\geqslant$50% PSA decline on enzalutamide.
Conclusion: Our preliminary case series data suggest limited activity of enzalutamide in the post-docetaxel and post-abiraterone patient population.
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1. Introduction

In the last 2 years two novel androgen-targeting agents have shown improved overall survival (OS) and quality of life for men with advanced prostate cancer [1,2]. The CYP17 inhibitor abiraterone and the novel anti-androgen enzalutamide were approved by the US Food and Drug Administration (FDA) in April 2011 and August 2012 respectively, for men with castration-resistant prostate cancer (CRPC) after docetaxel chemotherapy.

Abiraterone is an irreversible and potent inhibitor of CYP17, a key enzyme for the extragonadal synthesis of androgens and estrogens. In the post-docetaxel Phase III COU-AA-301 study, abiraterone with prednisone demonstrated a 4.6-month increase in OS, with improvements in all secondary end-points, compared to placebo with prednisone [1]. Enzalutamide is a next generation non-steroidal anti-androgen that was compared to placebo in the Phase III AFFIRM trial and demonstrated a 4.8-month improvement in median OS along with superiority in all secondary efficacy measures [2]. On the COU-AA-301 trial prior enzalutamide treatment was excluded and, likewise, on the AFFIRM study prior abiraterone was not allowed. Therefore, although the individual efficacy of abiraterone and enzalutamide after docetaxel is well established, the therapeutic benefit of targeting androgen receptor (AR) signalling by sequential administration of these agents is not clear. We, and others, have reported retrospective data showing that abiraterone has limited activity when used after docetaxel and enzalutamide [3,4].

Several mechanisms of intrinsic or acquired resistance to abiraterone have been proposed *in vitro* and *in vivo* [5]. Furthermore, additional lines of therapy may allow sequential acquisition of mutations and clonal evolution that may impact the activity of subsequent treatments. The aim of this study was to retrospectively analyse the antitumour activity and safety of enzalutamide in patients previously treated with docetaxel and abiraterone.

2. Patients and methods

2.1. Eligibility

Patients with metastatic CRPC that started treatment with enzalutamide within an Expanded Access Programme (EAP) at the Royal Marsden (RM) and Guy's and St. Thomas' (GS) NHS Foundation Trusts between June 2012 and February 2013 were retrospectively identified. Clinical data were collected from the electronic patient record. Criteria for inclusion in this study were histological diagnosis of prostate adenocarcinoma, previous docetaxel and abiraterone treatment, ongoing androgen deprivation therapy, Eastern Cooperative Group Performance Status (ECOG PS) 0–2, adequate haematological, hepatic and kidney function and the absence of brain metastases or a history of seizures. Patients recruited at the RM signed an informed consent allowing data collection. Local ethical approval was granted for the collection of clinical data of the patients recruited at GS.

2.2. Treatment plan and evaluations

Enzalutamide was administered orally at a dose of 160 mg once daily. Baseline evaluations included medical history and physical exam, baseline imaging assessments with computed tomography (CT) and bone scans, and baseline prostate specific antigen (PSA) levels. Clinical assessments with PSA levels and blood tests including full blood count and hepatic and renal function were performed every 4 weeks. Radiological assessments were performed approximately every 6 months. Dose reductions by 25% or 50% (120 or 80 mg once daily) were allowed in case of toxicity.

2.3. Outcome measures and statistical analysis

Descriptive analyses were performed to determine progression free survival (PFS) and OS, rates of PSA declines and factors associated with response and survival. PSA response was defined as a PSA decline by $\geqslant 50\%$ from baseline and PSA progression as PSA rise by 25% from the nadir, both confirmed with a second PSA value at least three weeks later. Radiological response and progression were evaluated according to PCWG2 (Prostate Cancer Working Group criteria 2) and to RECIST 1.1 (Response Evaluation Criteria in Solid Tumours 1.1) [6,7] for patients with measurable disease. Clinical progression was a composite end-point that included increased disease related pain (assessed according to the 11-point numeric rating scale, with 0 representing the absence of pain and 10 representing the worst imaginable pain [8]), skeletal related events (SREs), indication for palliative radiotherapy or cancer related worsening of ECOG PS. Adverse events were graded using common terminology criteria for adverse events (CTCAE) v4.0. OS was defined as the interval between start of enzalutamide and death or date of last follow-up. PFS was defined as the interval from initiation of enzalutamide and the date of PSA, radiological or clinical progression. Time to PSA progression was defined as the interval between the initiation of enzalutamide and the date of PSA progression according to the PCWG2 criteria. Time on treatment was defined as the time interval between the date of first and last intake.

Statistical analysis was performed with SPSS Statistics v20 (IBM), using the 2nd May 2013 as the cut-off date. Duration of treatment, OS, PFS and PSA progression were calculated using Kaplan-Meier estimates. Patients still on treatment or alive were censored. The

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association between PSA response on enzalutamide and OS, and of previous response to abiraterone and PFS on enzalutamide were explored using Cox regression models. The association of rates of ⩾50% PSA response on previous abiraterone with rates of ⩾50% PSA responses on enzalutamide was explored using binary logistic regression models.

3. Results

3.1. Patients characteristics

Between June 2012 and February 2013 a total of 39 patients were identified. The patient characteristics at abiraterone and enzalutamide initiation are described in Tables 1 and 2 respectively. The response and duration of abiraterone treatment are described in Table 3. Of the 38 patients who received abiraterone after docetaxel, 35 received abiraterone within the NHS (National Health System) and three were treated within clinical trials (two patients within the Phase I–II COU-AA-003 trial and one patient within the COU-AA-BE trial). One patient participated in the Phase III COU-AA-302 trial and received abiraterone prior to docetaxel. The most common metastatic sites were bone and lymph nodes. The majority of patients had ECOG PS 1 (61.5% at the start of abiraterone and 64.1% at the start of enzalutamide) with an increased proportion of patients with ECOG PS 2 at the time of enzalutamide initiation (7.6% at the start of abiraterone and 35.8% at the start of enzalutamide). Most patients (76.9%) started enzalutamide with symptoms of disease related pain and had already received several lines of hormonal therapies and chemotherapies. A total of 14 of 39 patients (35.8%) had received also cabazitaxel as second-line chemotherapy.

A total of 25 patients were already on treatment with low dose steroids (10 mg of prednisolone or 0.5 mg of dexamethasone) at start of enzalutamide and six patients required an increase in steroid dose while on treatment due to worsening fatigue and/or anorexia. Three patients had steroids prescribed during treatment with enzalutamide for the same indications. The median follow-up was 4.3 months (range: 1.0–8.2 months).

Table 1
Patient characteristics at initiation of abiraterone treatment.

		$N = 39$	%
Age (years)	Median	68	
	Range	54–85	
ECOG PS	0	6	15.3%
	1	24	61.5%
	2	3	7.6%
	Unknown	6	15.3%
Gleason score	⩾8	21	53.8%
	=7	10	25.6%
	⩽6	7	17.9%
	NA	1	2.5%
Number of previous hormonal therapies	Median	3	
	Range	1–5	
Bicalutamide	Yes	35	89.7%
	No	4	10.3%
Docetaxel number of cycles	Median	8	
	Range	1–12	
PSA response to docetaxel	No	13	33.3%
	Yes	20	51.3%
	⩾30% and <50%	5	12.8%
	⩾50% and <90%	9	23.1%
	⩾90%	6	15.4%
	NA	6	15.4%
Metastatic sites	Bone	33	84.6%
	Nodes	18	46.1%
	Visceral	4	10.2%
PSA (μg/L)	Median	222	
	Range	2.3–997	
Hb (g/dL)	Median	11.8	
	Range	8.1–14.7	
LDH (UI/L)	Median	192	
	Range	14.7–508	
ALP (UI/L)	Median	93.5	
	Range	23–1580	
Albumin (g/L)	Median	40	
	Range	31–50	

ECOG PS = Eastern Cooperative Oncology Group Performance Status; NA = not available; PSA = prostate specific antigen; Hb = haemoglobin; LDH = lactate dehydrogenase; ALP = alkaline phosphatase.

3.2. Response to enzalutamide

At the time of the analysis nine patients remain on enzalutamide treatment, all of which have received at least 12 weeks of continuous treatment. The other 30 patients have discontinued enzalutamide, 28 (93.3%) due to disease progression; one because of side-effects (G3 fatigue and G1 skin rash); and one because of G3 acute kidney failure related to progressive disease diagnosed 6 days after the start of treatment and requiring discontinuation after 22 days of treatment. A total of 20 patients (20/39, 51.3%) discontinued enzalutamide in the first 3 months of treatment due to disease progression.

Overall 16 patients (41.1%; 95% confidence interval (CI) 27.1% to 56.6%) had a confirmed PSA decline on enzalutamide of at least 30%. A confirmed PSA decline of ⩾50% and ⩾90% was achieved in 5/39 (12.8%; 95% CI 5.6% to 26.7%) and 1/39 (2.6%; 95% CI 0.4% to 13.2%) of patients respectively. Of the six patients with a confirmed PSA decline of at least

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Table 2
Patient characteristics at initiation of enzalutamide treatment.

		$N = 39$	%
Age (years)	Median	70	
	Range	54–85	
Type of post-abiraterone treatment	Cabazitaxel	14	35.8
	None	15	38.4
	Docetaxel retreatment	1	2.5
	Others	9	23
ECOG PS	0	0	0
	1	25	64.2
	2	14	35.8
Disease related pain	0	9	23.1
	1–3	17	43.6
	4–6	10	25.6
	7–10	3	7.7
PSA (μg/L)	Median	500	
	Range	15–6357	
Metastatic sites	Bone	33	84.6
	Nodes	21	53.8
	Visceral	6	15.3
Hb (g/dL)	Median	11	
	Range	8.3–14.2	
LDH (UI/L)	Median	225	
	Range	86–876	
ALP (UI/L)	Median	101	
	Range	29–2066	
Albumin (g/L)	Median	37	
	Range	24–48	

ECOG PS = Eastern Cooperative Oncology Group Performance Status; PSA = prostate specific antigen; Hb = haemoglobin; LDH = lactate dehydrogenase; ALP = alkaline phosphatase.

50%, four had received two previous lines of anti-androgen therapy and the other two received three previous anti-androgen therapies. All six patients had received previous bicalutamide, with duration of responses that ranged from 2 to 10 months. Four of the six patients were receiving steroids at the initiation of enzalutamide treatment.

An additional four patients (10.3%; 95% CI 4.1% to 23.6%) had an unconfirmed PSA decline of at least 50%; in three of these patients the response was short lived and in one, treatment is still ongoing (Table 4). A waterfall plot of maximal PSA changes is shown in Fig. 1. A 50% PSA response was achieved in 4 out of 28 (14.3%; 95% CI 5.7% to 31.5%) patients with, and in 4 out of 11 (36.4%; 95% CI 15.2% to 64.6%) patients without, concomitant steroid treatment ($p = 0.15$).

Of 23 patients with measurable disease, eight patients (34.8%; 95% CI 18.8% to 55.2%) had progressive disease, four (17.4%; 95% CI 7% to 37.1%) remained radiologically stable and one patient (4.3%; 95% CI 0.8% to

Table 3
Response to abiraterone treatment.

Patients	$N = 39$	N (%)
PSA decline on abiraterone	$\geqslant$30% confirmed	19 (48.7%)
	$\geqslant$50% total	15 (38.4%)
	$\geqslant$50% confirmed	15 (38.4%)
	$\geqslant$90%	6 (15.3%)
	No	18 (46.2%)
	NE	2 (5.1%)
Treatment duration on abiraterone (months)	Median	6.4
	95% CI	3.6–9.2
Reason for discontinuation	Progression	38 (97.4%)
	Radiological	17 (44.7%)
	Biochemical	35 (92.1%)
	Clinical	17 (44.7%)
	Toxicity*	1 (2.5%)

NE = not evaluable; PSA = prostate specific antigen; CI = confidence interval.
* One patient discontinued abiraterone due to G3 alanine aminotransferase (ALT) elevation.

21%) had a partial response. The remaining 10 patients were either discontinued due to clinical progression or were still on treatment at the time of the analysis but not yet assessed radiologically. The median duration of treatment with enzalutamide was 2.9 months (95% CI 1.7–4.0 months). 17 patients (43.6%; 95% CI 29.3% to 59%) were on treatment for at least 3 months, and four patients (10.3%; 95% CI 4.1% to 23.6%) received treatment for longer than 6 months; two of these patients are still on treatment with enzalutamide. Median OS was not reached at the time of data cut-off, with only eight events at the time of analysis. The median PFS was 2.8 months (95% CI 2.0–3.6 months) (Fig. 2). Median time to PSA progression was 2.7 months (95% CI 2.5–3 months).

3.3. Response to enzalutamide with respect to previous abiraterone therapy

To evaluate whether previous response to abiraterone could predict response to enzalutamide we analysed the response to enzalutamide in abiraterone-responders (patients with a confirmed PSA decline of at least 50%) and in abiraterone-refractory patients (patients with a PSA decline of less than 50%). Of the 39 patients, 15 (38.5%; 95% CI 24.9% to 54.1%) had had at least a confirmed $\geqslant$50% PSA decline on abiraterone. In two patients the PSA response on abiraterone was not evaluable due to missing data. On subsequent enzalutamide treatment seven of the fifteen abiraterone-responders

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Table 4
Response to enzalutamide treatment ($N = 39$ unless otherwise specified).

		N	% (95% CI)
Current patients status (as at 02/05/2013)	Ongoing	9	23.1%
	Discontinued	30	76.9%
PSA decline on enzalutamide	⩾30% confirmed	16	41.1% (27.1–56.6)
	⩾50% total	9	23% (12.6–38.3)
	⩾50% confirmed	5	12.8% (5.6–26.7)
	⩾90%	1	2.6% (0.4–13.2)
	No	22	56.4% (41–70.7)
	NE**	1	2.6% (0.4–13.2)
Radiological response	Measurable disease	23/39	58.9% (43.4–72.9)
	PR	1/23	4.3% (0.8–21)
	SD	4/23	17.4% (7–37.1)
	PD	8/23	34.8% (18.8–55.2)
	NE	10/23	43.4% (25.6–63.2)
Treatment duration (months)	Median (95% CI)	2.9 m	(1.7–4.0)
	Range	0.6–7.2 m	
	>3 months*	17 (9 ong)	43.6% (29.3–59)
	>6 months	4 (2 ong)	
Reason for discontinuation	Disease progression	26	92.8% (77.4–98)
	Radiological	10/26	38.5% (22.4–57.5)
	Biochemical	19/26	73.1% (53.9–86.3)
	Clinical	22/26	84.6% (66.5–93.9)
	Toxicity**	1	3.6% (0.6–17.7)
	Other***	1	3.6% (0.6–17.7)
OS (months)	Median	NR	
PFS (months)	Median	2.8 m	(2–3.6)
	Range	2.0–3.7	

PR = partial response; SD = stable disease; PD = progressive disease; NE = not evaluable; m = months; ong = ongoing; PSA = prostate specific antigen; CI = confidence interval.
* Includes the four patients with a treatment duration >6 months.
** One patient was discontinued due to G1 skin rash and G3 fatigue, enzalutamide related.
*** One patient discontinued enzalutamide after 22 days due to G3 acute renal failure, disease related, not enzalutamide related.

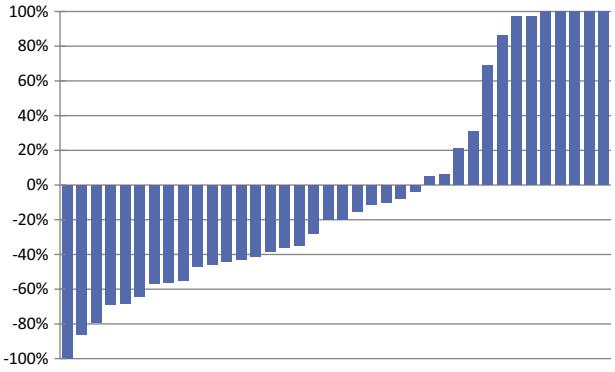

Fig. 1. Maximum prostate specific antigen (PSA) decline on enzalutamide in all patients (PSA increase capped at 100%).

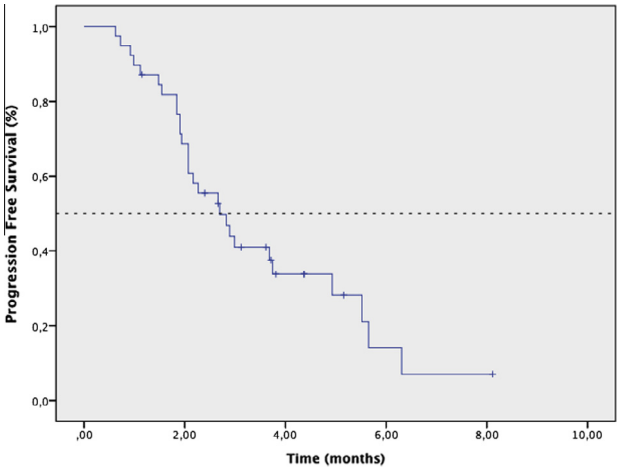

Fig. 2. Progression free survival on enzalutamide.

(46.7%; 95% CI 24.8% to 69.9%) achieved a confirmed ⩾30% PSA decline while two patients (13.3%; 95% CI 3.7% to 37.9%) achieved a confirmed ⩾50% PSA decline (Fig. 3). Of the 22 patients with a PSA decline of less than 50% on abiraterone eight patients (36.4%; 95% CI 19.7% to 57%) had a confirmed ⩾30% PSA decline on enzalutamide with two patients (9.1%; 95% CI 2.5% to 27.8%) reaching a confirmed ⩾50% PSA decline (Fig. 4); one of these biochemical responses is still ongoing. There was no association between previous ⩾50% PSA response to abiraterone and ⩾50% PSA response to enzalutamide ($p = 0.186$). There was no

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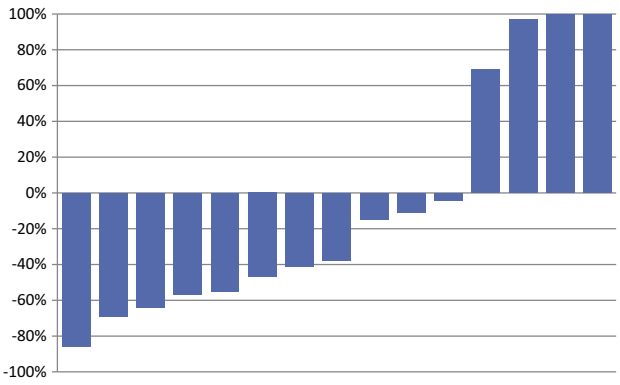

Fig. 3. Maximum prostate specific antigen (PSA) decline on enzalutamide in patients with >50% PSA decline on previous abiraterone (PSA increase capped at 100%).

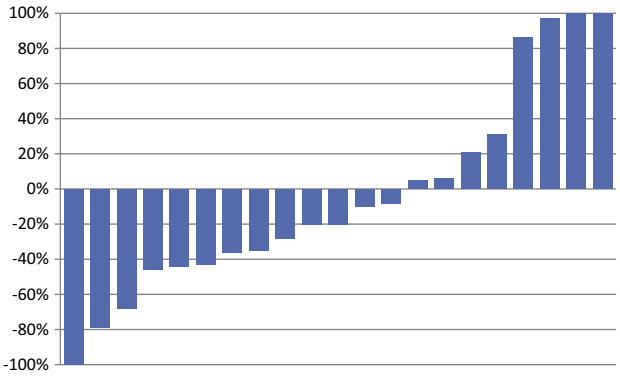

Fig. 4. Maximum prostate specific antigen (PSA) decline on enzalutamide in patients with <50% PSA decline on previous abiraterone (PSA increase capped at 100%).

statistically significant difference in PFS on enzalutamide in previous abiraterone-responders compared with non-responders (2.99 versus 2.27 months, $p = 0.76$).

3.4. Safety

The main side-effect of enzalutamide treatment reported in 64.1% of patients was fatigue (all grades). In the majority of cases (38.4%) fatigue was of moderate severity (G2), but fatigue was severe (G3) in one patient (2.5%) and led to permanent treatment discontinuation. G2/3 fatigue required temporary treatment interruption in five patients; in two additional patients G2 fatigue was managed with a dose reduction by 25% and 50% respectively. Other common side-effects included anorexia (20.5%, all grades), nausea (10.2%, all grades) and depressive mood (7.6%, all grades). No seizures were reported.

4. Discussion

With the successful development and approval of cabazitaxel, abiraterone and enzalutamide physicians are facing the difficult task of selecting the optimal sequence for patients with CRPC progressing post-docetaxel. At present, no clinical or biological factors have been shown to predict response to these agents and treatment choices are based on availability, patient and clinician preferences and performance status or comorbidities.

This retrospective study provides for the first time preliminary data on the activity of enzalutamide in advanced and heavily pre-treated CRPC patients previously treated with abiraterone. In these patients, enzalutamide resulted in confirmed $\geqslant$50% PSA declines in 12.8% of patients. The median duration of treatment was 2.9 months (95% CI 1.7–4). In patients deemed abiraterone-refractory on the basis of <50% PSA decline, we observed $\geqslant$30% and $\geqslant$50% PSA declines on enzalutamide in eight (36.3%) and three patients (13.6%) respectively. Similarly, a recently presented phase I/II study of ARN-509, a novel AR antagonist with a similar mechanism of action to enzalutamide, reported a considerably lower 50% PSA decline rate in abiraterone pre-treated patients (4 out of 14, 29%), than in abiraterone-naïve patients (22 out of 25, 88%) [9]. These data indicate some cross-resistance between these novel agents.

Our dataset was limited by the small patient population as well as the retrospective nature of the analysis. Despite these limitations, we observed lower rates of PSA declines and shorter durations of enzalutamide treatment in the post-docetaxel and post-abiraterone setting compared with the PSA response rate of 54% and median treatment duration of 8.3 months reported in the AFFIRM study in abiraterone-naïve patients [2] and the even higher response rates seen in docetaxel naïve patients. These differences may have been influenced by the advanced disease stage in our population (ECOG PS 2 in 8.5% of patients enrolled in AFFIRM versus 35.8% in our cohort) resulting in reduced physical tolerance and worse outcomes. Nevertheless, any mechanisms of cross-resistance between abiraterone and enzalutamide appear incomplete and included a 99.7% confirmed PSA decline and long-lasting response to enzalutamide in one patient whose disease had proved refractory to abiraterone.

Similarly, our recently published data on the activity of abiraterone in 38 post-docetaxel and post-enzalutamide patients showed confirmed $\geqslant$50% PSA declines in only 8% of patients and a median PFS of 2.7 months (95% CI: 2.3–4.1) [4], which was inferior to the PSA response rate of 29% and median treatment duration of 8 months for enzalutamide-naïve patients reported in the COU-AA-301 trial [1].

Preclinical data have proposed several possible mechanisms of resistance to abiraterone and enzalutamide, including AR mutations [5,10], constitutively active AR splice variants [11], AR activation by concomitant

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glucocorticoid use [12,13] and activation of AR-independent signalling pathways such as the phosphatidylinositide 3-kinase (PI3K) - protein kinase B (Akt) - mammalian target of rapamycin (mTOR) pathway [14]. Several mechanisms of resistance are likely in different patients, and perhaps even in the same patient, due to clonal heterogeneity as a result of disease clonal evolution induced by therapeutic selective pressure.

Overall, enzalutamide remained a safe and relatively well tolerated treatment in an advanced patient population. In the absence of better clinical or biological predictive factors of treatment response, the activity of enzalutamide after docetaxel and abiraterone may represent a valid treatment option for the advanced prostate cancer patient.

In conclusion, enzalutamide has modest antitumour activity in advanced-stage CRPC patients when used after docetaxel and abiraterone. Larger prospective studies are now needed to provide further data regarding optimal treatment sequencing and the potential benefits of combing abiraterone and enzalutamide.

Conflict of interest statement

Disclosure: Abiraterone acetate was developed at The Institute of Cancer Research, which therefore has a commercial interest in the development of this agent.

C.P. received lecture fees from Sanofi-Aventis and travel support from Sanofi-Aventis and Janssen-Cilag. G.A. received consulting fees and travel support from Janssen-Cilag, Veridex, Roche/Ventana and Millennium Pharmaceuticals, lecture fees from Janssen-Cilag, Ipsen, Takeda and Sanofi-Aventis, and grant support from AstraZeneca and Genentech. G.A. is on The ICR rewards to inventors list of abiraterone acetate. S.C. received honoraria and is a member of the advisory boards of Janssen-Cilag and Sanofi-Aventis. J.S.d.B. received consulting fees from Ortho Biotech Oncology Research and Development (a unit of Cougar Biotechnology), consulting fees and travel support from Amgen, Astellas, AstraZeneca, Boehringer Ingelheim, Bristol-Myers Squibb, Dendreon, Enzon, Exelixis, Genentech, GlaxoSmithKline, Medivation, Merck, Novartis, Pfizer, Roche, Sanofi-Aventis, Supergen, and Takeda, and grant support from AstraZeneca and Genentech.

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7.3 Is there an antiandrogen withdrawal syndrome with enzalutamide? (90)

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Is there an antiandrogen withdrawal syndrome with enzalutamide?

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Objective

To examine prostate-specific antigen (PSA) levels after enzalutamide discontinuation to assess whether an antiandrogen withdrawal syndrome (AAWS) exists with enzalutamide.

Methods

We retrospectively identified 30 consecutive patients with metastatic prostate cancer who were treated with enzalutamide after docetaxel. Post-discontinuation PSA results were available for all patients and were determined at 2-weekly intervals until starting further anticancer systemic therapy. PSA withdrawal response was defined as a PSA decline by ≥50% from the last on-treatment PSA, with a confirmed decrease ≥3 weeks later. Patient characteristics were evaluated in relation to the AAWS using univariate logistic regression analysis.

Results

The median (range) patient age was 70.5 (56–86) years and the median (range) follow-up was 9.0 (0.5–16) months. The most common metastatic sites were the bone (86.7%) and lymph nodes (66.7%). Most patients (70%) had previously received abiraterone and 12 patients (40%) had also received cabazitaxel. The median (range) treatment duration with enzalutamide was 3.68 (1.12–21.39) months. PSA levels after enzalutamide withdrawal were monitored for a median (range) time of 35 (10–120) days. Only one patient (3.3%) had a confirmed PSA response ≥50% after enzalutamide discontinuation. One patient (3.3%) had a confirmed PSA response of between 30 and 50% and another patient (3.3%) had an unconfirmed PSA response of between 30 and 50%. The median overall survival was 15.5 months (95% CI 8.1–24.7). None of the factors analysed in the univariate analysis were significant predictors of PSA decline after enzalutamide discontinuation.

Conclusions

This retrospective study provides the first evidence that enzalutamide may have an AAWS in a minority of patients with metastatic castration-resistant prostate cancer. Further studies are needed to confirm the existence of an enzalutamide AAWS and to assess its relevance in prostate cancer management.

Keywords

castration-resistant prostate cancer, enzalutamide, antiandrogen withdrawal syndrome, hormone therapy, androgen receptor targeting, androgen receptor gene F876L mutation

Introduction

Prostate cancer is the most common cancer in men, with an estimated 382 000 cases occurring in Europe during 2008, and is the third most common cause of death from cancer in men [1]. Primary androgen deprivation therapy with LHRH analogues is the standard of care first-line treatment in patients with metastatic prostate cancer; however, most patients will ultimately experience disease progression at a median of 18–24 months after commencing androgen deprivation therapy, developing castration-resistant prostate cancer (CRPC) [2,3]. Despite the lack of level one evidence, secondary hormonal manipulations, such as the addition of an antiandrogen to achieve combined androgen blockade, has long been the most used second-line hormone treatment in patients with metastatic prostate cancer progressing on androgen deprivation therapy. For those patients who later progress whilst receiving an antiandrogen, discontinuation of the antiandrogen has been reported to reduce PSA levels in 15–30% of patients [4–6] in what it is known as antiandrogen

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withdrawal syndrome (AAWS). The most accepted definition of AAWS is a decline in PSA level of ≥50% from baseline after antiandrogen cessation, with a confirmed decrease ≥3 weeks later [4].

In 1997, AAWS in prostate cancer was described for the first time after discontinuation of the non-steroidal antiandrogen flutamide [4–8]. Subsequently, similar withdrawal responses have been reported in patients treated with other non-steroidal antiandrogens, such as bicalutamide [4–6,9] and nilutamide [4,5,10,11], as well as with steroidal antiandrogens, such as cyproterone acetate [12] and megestrol acetate [13,14]. While symptomatic benefit and objective radiographic responses have been reported in some cases of AAWS, no impact on survival has ever been shown. After a median PSA response duration of 3.5–5.0 months, further anticancer treatment is generally required because of disease or PSA progression [5]. Nevertheless, AAWS is important in understanding the biology of the androgen receptor (AR) and the mechanisms leading to castration resistance, and still remains a common hormonal manipulation in daily practice.

The molecular mechanisms underlying AAWS have not been fully determined. The most accepted mechanism is AR gene mutations leading to alterations in the AR ligand-binding domain, which cause antiandrogens to act as partial agonists [15]. To date, several preclinical studies using prostate cancer cell models have shown that specific mutations in the AR gene are responsible for the switch of bicalutamide [16] and flutamide [17] from AR antagonist to partial agonist.

Enzalutamide is a second generation non-steroidal antiandrogen which significantly improves overall survival (OS) and progression-free survival (PFS) in patients with metastatic CRPC progressing after docetaxel. The enzalutamide postdocetaxel phase III trial [18] reported a 4.8-month improvement in OS and a 37% reduction in the risk of death for enzalutamide compared with placebo. That study led to the approval of enzalutamide in men with metastatic CRPC after docetaxel. While an AAWS has been reported for almost all other non-steroidal antiandrogens, there is no published evidence of any withdrawal effect with enzalutamide. Unlike bicalutamide and flutamide, no AR agonist effect was shown in enzalutamide preclinical studies [19]. Hence, given the fact that enzalutamide is a second-generation AR antagonist, it is believed that enzalutamide may not have an AAWS.

The aim of the present study was to examine PSA levels after enzalutamide discontinuation to assess whether an AAWS exists with enzalutamide and to correlate post-withdrawal PSA with survival. A secondary objective of the study was to assess patients' clinical and treatment factors associated with a potential AAWS using univariate logistic regression analyses.

Patients and Methods

Patients

Patients with metastatic CRPC treated with enzalutamide in the UK between June 2012 and September 2013 were included. Eligibility criteria for the present study were histologically confirmed metastatic adenocarcinoma of the prostate without neuroendocrine differentiation, ongoing androgen deprivation therapy or bilateral orchidectomy, previous treatment with docetaxel chemotherapy, Eastern Cooperative Group (ECOG) performance status score 0–2, adequate haematological, hepatic and kidney function and the absence of brain metastases or a history of seizures. All histological specimens were centrally reviewed and neuroendocrine differentiation was excluded using immunohistochemistry (markers: chromogranin A, synaptophysin and CD56). Patients with evidence of disease progression on enzalutamide were selected and their data were analysed from patient records. Only patients with at least one available PSA post-enzalutamide discontinuation were included. Concomitant treatment with zoledronic acid was allowed. Patients who received palliative radiotherapy immediately after enzalutamide cessation were excluded, as were patients on low-dose steroids.

Treatment

Enzalutamide was administered orally at a dose of 160 mg once daily. Dose reductions of 25 or 50% were allowed. Clinical assessments with medical history, physical examination, PSA levels and blood tests, including full blood count and hepatic and renal function, were performed at study entry and at 4-weekly intervals thereafter. Baseline imaging assessments included chest, abdomen and pelvis CT and bone scans. Radiological assessments were performed according to standard of care approximately every 3–6 months. After enzalutamide discontinuation, clinical assessment and PSA tests were performed every 2 weeks until further anticancer treatment was started. CT and bone scans were performed 4 weeks after enzalutamide cessation when possible.

Clinical Outcomes

PSA changes while on treatment were assessed according to the Prostate Cancer Working Group 2 criteria [20]. PSA response was defined as a PSA decline by ≥50% from baseline and PSA progression as a PSA rise by 25% from the nadir, both confirmed with a second PSA value at least 3 weeks later. Response Evaluation Criteria in Solid Tumours 1.1 [21] and Prostate Cancer Working Group 2 criteria were used to assess measurable disease response and progression. Disease progression on bone scan was defined, using Prostate Cancer Working Group 2 criteria, as the appearance of two new bone lesions. Clinical progression was a composite endpoint that included increased disease-related pain, skeletal-related events,

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indication for palliative radiotherapy or cancer-related worsening of ECOG performance status. After enzalutamide withdrawal, PSA response was defined as a PSA decline by ≥50% from the last on-treatment PSA, with a confirmed decrease ≥3 weeks later.

The interval between enzalutamide initiation and death from any cause was the definition for OS. PFS was defined as time from enzalutamide initiation to disease progression in bone or soft tissue, symptoms or death. Time on treatment was defined as the interval between date of first and last dose of enzalutamide.

Ethics Approval

This study was carried out in accordance with the Declaration of Helsinki for experiments involving humans. Local ethical approval was granted for the collection of patients' clinical data.

Statistical Analysis

Statistical analysis was performed with SAS Statistics, using 22 October 2013 as the cut-off date. OS and PFS were calculated using Kaplan–Meier estimates. The association between post-withdrawal PSA and OS was explored using Cox regression models. The prognostic association between clinical and treatment factors and a potential AAWS was studied using univariate logistic regression analyses.

Results

Patient Characteristics

Between June 2012 and October 2013, 30 consecutive patients treated with enzalutamide for metastatic CRPC were identified. The patient characteristics at enzalutamide initiation are summarised in Table 1. The median (range) age before starting enzalutamide was 70.5 (56–86) years. The majority of patients had an ECOG performance status score of 1 (70%). Six patients (20%) had an ECOG performance status score of 2. The most common metastatic sites were bone (86.7%) and lymph nodes (66.7%), and 30% of patients had visceral metastases. Eleven patients had had previous local treatment with either radical prostatectomy (two patients, 6.7%) or radical prostatic radiotherapy (nine patients, 30%). The median (range) number of previous systemic anticancer therapies was 5 (3–7), including hormone therapies. Most patients (70%) had received abiraterone previously and 40% of patients had also previously received cabazitaxel. Eight patients (26.7%) had previously received zoledronic acid (median [range] number of cycles 14 [2–23]). Enzalutamide was initiated because of clinical progression (83.3%), PSA progression (50%) and/or radiological progression (86.7%).

Table 1 Patient characteristics at enzalutamide initiation (n = 30).

Median (range) age, years	70.5 (56–86)
Ethnicity, *n* (%)	
White	24 (80.0)
Black	4 (13.3)
Asian	2 (6.7)
ECOG performance status, *n* (%)	
0	3 (10.0)
1	21 (70.0)
2	6 (20.0)
Gleason score, *n* (%)	
≤6	3 (10.0)
7	6 (20.0)
≥8	18 (60.0)
NA	3 (10.0)
Median (range) PSA, ng/mL	263.95 (15–6357)
Metastatic sites, *n* (%)	
Bone	26 (86.7)
Lymph node	20 (66.7)
Liver	5 (16.7)
Lung	2 (6.7)
Other	2 (6.7)
Previous local treatment, *n* (%)	
Radical prostatectomy	2 (6.7)
Radical radiotherapy	9 (30.0)
None	19 (63.3)
Previous systemic therapies, *n* (%)	
LHRH agonist	30 (100.0)
Bicalutamide	27 (90.0)
Steroids	11 (36.7)
Stilboestrol	15 (15.0)
Docetaxel	30 (100.0)
Abiraterone	21 (70.0)
Cabazitaxel	12 (40.0)
Zoledronic acid	8 (26.7)
ECarboF	2 (6.7)
Median (range) response to LHRH agonist, months	12.5 (3–119)
Reason for starting enzalutamide, *n* (%)	
Clinical progression	25 (83.3)
PSA progression	15 (50.0)
Radiological progression	26 (86.7)
Median (range) haemoglobin, g/dL	11.3 (8.4–14.4)
Median (range) albumin, g/L	41 (28–48)
Median (range) alkaline phosphatase, UI/L	263.95 (15–6357)

ECOG PS, Eastern Cooperative Oncology Group Performance Status; NA, not available; ECarboF, epirubicin, carboplatin and 5-fluorouracil.

Response to Enzalutamide

The median (range) duration of treatment with enzalutamide was 3.68 (1.12–21.39) months. A confirmed PSA decline of ≥50% from baseline was recorded in nine patients (30%, 95% CI 17–48 [Table 2]). Six patients (20%, 95% CI 8–39) had a confirmed PSA reduction of 30–50%. Overall, 15 patients (50%) achieved a confirmed PSA decline of at least 30%. Seven patients (23%) had no PSA reduction (PSA stabilization or increase). The median PSA was 263.95 before enzalutamide initiation and 113.6 at nadir on enzalutamide (22% reduction in median PSA, $P = 0.017$). Among patients with a confirmed PSA decline of ≥50%, all had received previous bicalutamide, five patients had received two previous lines of antiandrogen therapy and one patient had received three previous lines of antiandrogen therapy. Four

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Table 2 Response to enzalutamide (*N* = 30).

Median (range) treatment duration, months	3.68 (1.12–21.39)
Median (range) baseline PSA before enzalutamide initiation, ng/mL	263.95 (15.00–6357.00)
Median (range) PSA nadir onenzalutamide, ng/mL	113.65 (4.80–4586.00)
PSA decline onenzalutamide, *n* (%; 95% CI)	
≥50% confirmed	9 (30; 17–48)
30–50% confirmed	6 (20; 8–39)
<30%	8 (27; 15–43)
No PSA decline	7 (23; 12–41)
Radiological response on measurable disease*, *n* (%; 95% CI)	
Partial response	5 (26.3; 12–49)
Stable disease	3 (15.8; 6–38)
Disease progression	6 (31.6; 16–54)
NA	5 (26.3; 12–49)
Median (range) overall survival, months	15.5 (8.1478–24.7392)
Progression free survival, months	6.8 (2.5626–5.5195)

*N = 19. *NA, not available.*

Table 3 Patient characteristics at enzalutamide discontinuation (*N* = 30).

Median (range) PSA at enzalutamide discontinuation, ng/mL	235.70 (12.00–5718.00)
Reason for discontinuation, *n* (%)	
Disease progression	28 (93.3)
Clinical progression	22 (73.3)
PSA progression	27 (90.0)
Measurable disease progression	12 (40.0)
Bone scan progression	6 (20.0)
Toxicity	2 (6.7)
PSA decline after enzalutamide discontinuation, *n* (%)	
30–50% unconfirmed	1 (3.3)
30–50% confirmed	1 (3.3)
≥50% confirmed	1 (3.3)
No PSA decline	26 (86.6)
Subsequent treatment lines after enzalutamide discontinuation, *n* (%)	
None	12 (40.0)
Abiraterone	6 (20.0)
Cabazitaxel	2 (6.7)
Docetaxel	4 (13.3)
Cabozantinib	1 (3.3)
Mitoxantrone	2 (6.7)
Stilboestrol	4 (13.3)
ECarbF	4 (13.3)
Patient status at study analysis, *n* (%)	
Alive	14 (46.7)
Deaths	16 (53.3)
Prostate cancer deaths	16 (53.3)

ECarboF, epirubicin, carboplatin and 5-fluorouracil.

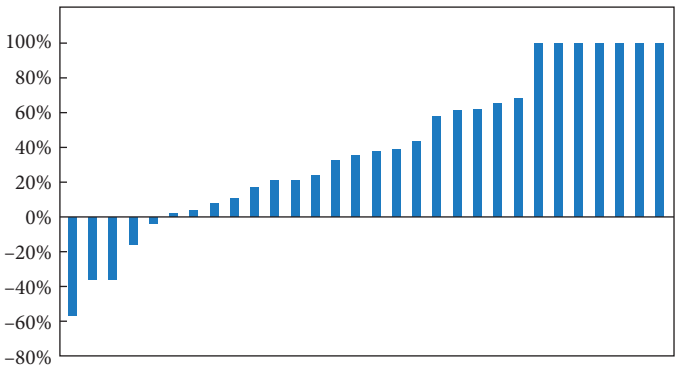
of them had previously received abiraterone and two had previously received cabazitaxel.

Amongst the 19 patients with radiologically measurable disease, five patients (26.3%, 95% CI 12–49) attained a partial response, three patients (15.8%, 95% CI 6–38) had disease stabilization and six patients (31.6%, 95% CI 16–54) had disease progression. The remaining five patients (26.3%, 95% CI 12–49) had treatment discontinued before being radiologically assessed because of clinical and biochemical progression (four patients) and bone scan progression (one patient). A radiological partial response was associated with a decline in ≥50% PSA in four patients. The median OS was 15.5 months (95% CI 8.1–24.7), the median PFS was 6.7 months (95% CI 2.6–5.5) and the median (range) follow-up was 9.0 (0.5–16) months.

PSA Levels after Enzalutamide Discontinuation

Patient characteristics at enzalutamide discontinuation are described in Table 3. Patients were withdrawn from enzalutamide because of clinical progression (73.3%), PSA progression (90%), radiological progression (60%) or toxicity (6.7%). The median (range) PSA at enzalutamide discontinuation was 235.7 (31.5–5718) ng/mL. PSA levels after enzalutamide withdrawal were monitored for a median (range) time of 35 (10–120) days, after which most patients (60%) started further systemic therapies. A second confirmatory PSA post-enzalutamide withdrawal value was available in 18 patients (60%). Only one patient (3.3%) had a confirmed PSA response of ≥50% after stopping enzalutamide. One patient (3.3%) had a confirmed PSA response of between 30 and 50% and another patient (3.3%) had an unconfirmed PSA response of between 30 and 50%. The remaining 27 patients (90%) had a raised PSA level after stopping enzalutamide. None of the three patients who experienced a

Fig. 1 Waterfall plot of PSA changes after enzalutamide discontinuation (PSA increase capped at 100%).



PSA reduction after enzalutamide discontinuation were receiving zoledronic acid. A waterfall plot of PSA changes after enzalutamide discontinuation is shown in Fig. 1. Amongst the 19 patients with radiologically measurable disease, post-withdrawal CT scans were available for seven patients (36.8%). No partial responses were seen, three patients showed stable disease and four patients had disease progression.

The characteristics of the three patients in whom an enzalutamide withdrawal effect was observed are summarised in Table 4. None of them underwent post-withdrawal CT. No

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Table 4 Characteristics of patients showing evidence of enzalutamide withdrawal syndrome.

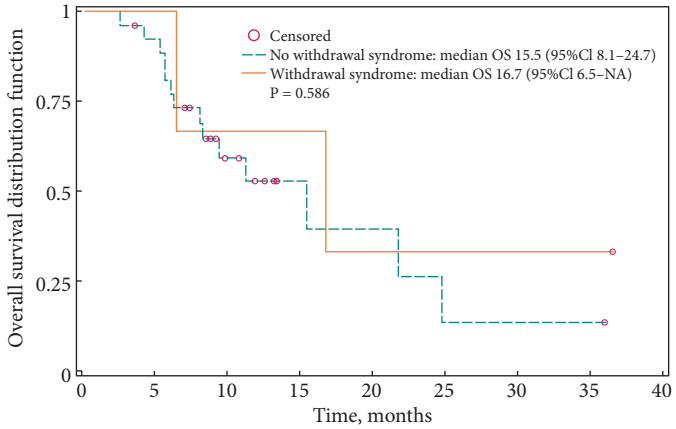
	Patient 1	Patient 2	Patient 3
Enzalutamide withdrawal effect	Confirmed PSA decline ≥50%	Confirmed PSA decline of 30–50%	Unconfirmed PSA decline of 30–50%
Time between enzalutamide discontinuation and PSA decline, days	40	35	50
Age at diagnosis of prostate cancer, years	49	65	68
Staging at diagnosis of prostate cancer	T3N0M0	T4N0M1	TxN0M0
Presenting PSA, ng/mL	61	2.023	150
Gleason score	10	9	7
Metastatic sites at diagnosis	Bone and lymph nodes	Bone only	Bone and lymph nodes
Duration of response to LHRH analogues, months	5	13	106
Previous treatment lines received	Bicalutamide, stilboestrol, docetaxel	Bicalutamide, stilboestrol, docetaxel	Bicalutamide, docetaxel, abiraterone
Age at diagnosis of metastatic prostate cancer, years	56	65	80
PSA decline on enzalutamide	Confirmed PSA response ≥50%	No PSA response	No PSA response
Radiological response to enzalutamide	Partial response	No measurable disease	NA
Radiological response after enzalutamide discontinuation	NA	No measurable disease	NA
Enzalutamide treatment duration, months	21.4	4.2	1.2

NA, not available.

symptomatic improvement was noted during enzalutamide AAWS. The patient who achieved a confirmed PSA decline of ≥50% after enzalutamide discontinuation had had a prolonged duration of treatment with enzalutamide (21.4 months). The AAWS occurred 40 days (5.7 weeks) after stopping enzalutamide. He had also had a confirmed PSA response of ≥50% during enzalutamide therapy, as well as a radiological partial response. His Gleason score was 10 and his presenting PSA was 61 ng/mL when first diagnosed with prostate cancer. His response to LHRH analogues lasted 5 months. He had received previous treatment with bicalutamide but did not have a bicalutamide AAWS.

There was a nonsignificantly longer median OS among patients with some enzalutamide AAWS (16.7 months, 95% CI 6.5–NA) as compared with patients with rising PSA after enzalutamide cessation (15.5 months, 95% CI 8.1–24.7; $P = 0.586$ [Fig. 2]).

Fig. 2 Kaplan–Meier plot for overall survival (OS) depending on enzalutamide withdrawal status. NA, not available.



Univariate Logistic Regression Analysis

The factors associated with enzalutamide AAWS were examined in a univariate analysis (Table 5). None of the factors analysed were statistically significant predictors of PSA decline after enzalutamide discontinuation. Patients who responded for longer to LHRH analogues had a borderline significantly higher probability of enzalutamide AAWS compared with those who progressed faster on LHRH analogues (odds ratio 1.02, 95% CI 0.98–1.05).

Discussion

The present study provides the first evidence that enzalutamide may have an AAWS in a minority of patients with metastatic CRPC. Although only one patient (3.3%) met the accepted criterion for AAWS, a confirmed PSA decline of ≥50%, three out of 30 patients (10%) showed a PSA decline of at least 30% after enzalutamide discontinuation. These data indicate that an AAWS is a possible yet rare event with enzalutamide. To our knowledge, this is the first study to examine PSA levels after enzalutamide cessation in patients with metastatic CRPC.

Despite the withdrawal PSA response observed in the present study, no symptomatic benefit or radiological responses were noted after enzalutamide withdrawal. Time between enzalutamide discontinuation and PSA response ranged between 35 and 50 days (5–7 weeks). The median OS time was slightly longer, although not statistically significant among patients with some enzalutamide AAWS as compared with patients with rising PSA after enzalutamide discontinuation (Fig. 2); however, the comparison of OS between groups is severely limited by the small sample size.

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Table 5 Logistic regression analysis for prediction of enzalutamide withdrawal syndrome.

Univariate predictor	Univariate OR (95% CI)
Ethnicity	
White	1.00 (Reference)
Black/Asian	2.20 (0.17–29.31)
Age at enzalutamide initiation (years)	0.97 (0.84–1.13)
PSA at enzalutamide initiation (ng/mL)	1.00 (1.00–1.00)
Metastatic sites at enzalutamide initiation	
Bone	1.00 (reference)
Lymph node	1.23 (0.10–15.87)
Visceral	NA
Type of progression on enzalutamide	
Clinical progression	1.00 (reference)
PSA progression	NA
Radiological progression	0.70 (0.06–8.82)
ECOG performance status	0.17 (0.01–1.93)
Haemoglobin (g/dL)	0.70 (0.32–1.55)
Albumin (g/L)	1.07 (0.84–1.36)
Alkaline phosphatise (UI/L)	1.00 (1.00–1.00)
Prior local treatment to the prostate	
Radical local treatment	1.18 (0.09–14.69)
None	1.00 (reference)
Number of previous treatment lines	1.89 (0.20–17.99)
Treatment duration with enzalutamide	1.12 (0.93–1.35)
Gleason score	
<8	10.73 (0.06–9.04)
8+	1.00 (reference)
Reason for enzalutamide discontinuation	
Bone scan/radiological progression	1.00 (reference)
Clinical progression	NA
PSA progression	NA
Toxicity	NA
Radiological response to enzalutamide	
Complete or partial response	NA
Stable disease	1.00 (reference)
Disease progression or unkown	NA
PSA decline >30% on enzalutamide	NA
Response to LHRH agonist (months)	1.02 (0.98–1.05)

ECOG, Eastern Cooperative Oncology Group; NA, not available.

Interestingly, both patients in whom a confirmed PSA response (Table 4) was observed had pretreatment features of aggressive prostate cancer, such as high Gleason score, high presenting PSA level and short response to LHRH analogues. The patient who achieved a confirmed PSA response ≥50% had a prolonged duration of treatment with enzalutamide (21.4 months). Moreover, he had also had a confirmed PSA response of ≥50% during enzalutamide therapy as well as a radiological partial response. Taken together, this information suggests that patients who respond well to enzalutamide and have longer durations of treatment may be more likely to experience an AAWS, similarly to what has already been described for bicalutamide [14]; however, none of the factors examined in our univariate analysis, including enzalutamide treatment duration, were statistically significant predictors of PSA decline after enzalutamide cessation (Table 5). Interestingly, patients who responded for a longer time to LHRH analogues had a borderline significantly higher probability of enzalutamide AAWS compared with those who progressed faster on LHRH analogues (odds ratio 1.02, 95% CI 0.98–1.05). The limited number of patients, as well as the low number of AAWS events, might partially explain why the logistic regression analyses failed to show any significant predictive factor for AAWS.

The present study has several limitations. The data are limited by the small sample size and by the retrospective nature of the study. The likelihood of detecting an enzalutamide withdrawal syndrome was limited by the fact that only 60% patients had a second confirmatory PSA test after enzalutamide discontinuation. The remaining 40% of patients started subsequent systemic therapies before having a second post-withdrawal PSA measured. Similarly, enzalutamide withdrawal syndrome may have been underestimated by the relatively short period of PSA monitoring after enzalutamide withdrawal (median [range] time 35 [10–120] days). While PSA responses after flutamide withdrawal usually appear within the first few days, bicalutamide withdrawal syndrome tends to occur 4–8 weeks after treatment cessation [6]. It is believed that this different pattern of PSA response is attributable to the long half-life of bicalutamide (half-life 5.9 days) [22] as compared with flutamide (half-life 6 h) [23]. Interestingly, enzalutamide has a half-life similar to bicalutamide (half-life 5.8 days) [24]; therefore, to assess a potential AAWS with enzalutamide, PSA levels should ideally be measured until 8 weeks after treatment discontinuation. In the present study, however, because 60% of patients started further systemic therapies, PSA levels were only monitored for a median time of 35 days (5 weeks). As AAWS has been described more frequently in patients with early-stage metastatic prostate cancer, the possibility of an enzalutamide AAWS may have been reduced because of the heavily pretreated status of the study population.

Importantly, three recently published preclinical studies have shown for the first time that specific AR mutations can confer partial agonist activity to enzalutamide in *in vitro* and *in vivo* models of CRPC. Balbas et al. [25] were the first to identify that prolonged treatment with enzalutamide in prostate cancer cell lines led to the spontaneous emergence of an AR novel specific mutation called F876L, which converted enzalutamide into an AR agonist. Moreover, structural analysis of the mutated AR showed that F876L mutation affects the ligand-binding domain of the AR and is responsible for the switch of enzalutamide from AR antagonist to AR agonist. A similar study by Joseph et al. [26] showed that F876L mutation was sufficient to convey acquired resistance not only to enzalutamide, but also to another second-generation antiandrogen, ARN-509, in CRPC cell models. Finally, another preclinical study by Korpal et al. [27] showed that F876L-bearing prostate cancer cells are not only resistant to enzalutamide, but also dependent on the agonist effect of enzalutamide for cellular growth under androgen-deprivation conditions. In view of this addiction phenomenon to agonist

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enzalutamide, the authors hypothesize that patients bearing the F876L-mutated AR might clinically benefit from withdrawal of enzalutamide.

These findings suggest that resistance to second-generation antiandrogens is mediated by the AR and therefore progressive CRPC is still an AR-driven disease, despite resistance to several hormone therapies. These studies provide a plausible molecular mechanism to explain the enzalutamide withdrawal syndrome seen in the present study. Second-generation antiandrogen withdrawal syndrome is therefore a new entity, the study of which may improve our understanding of resistance mechanisms and may help in the development of next-generation AR-targeted therapy.

In conclusion, the present preliminary study suggests that enzalutamide may have an AAWS in a minority of patients. Further studies are needed to confirm the existence of an enzalutamide AAWS and to assess its clinical relevance in prostate cancer management.

Conflict of Interest

S.C. has received honoraria and is a member of the advisory boards of Astellas, Janssen-Cilag, and Sanofi-Aventis. J.S.d.B. has received consulting fees from Ortho Biotech Oncology Research and Development (a unit of Cougar Biotechnology), consulting fees and travel support from Amgen, Astellas, AstraZeneca, Boehringer Ingelheim, Bristol-Myers Squibb, Dendreon, Enzon, Exelixis, Genentech, GlaxoSmithKline, Medivation, Merck, Novartis, Pfizer, Roche, Sanofi-Aventis, Supergen and Takeda, and grant support from AstraZeneca and Genentech. H.P. has attended and received honoraria for advisory boards, received travel expenses to medical meetings and served as a consultant for AstraZeneca, Astellas, Janssen, Sanofi Aventis, Takeda, Amgen, Ipsen, Ferring, Sandoz and Novartis. A.B. has received honoraria for advisory boards of Astellas, Janssen-Cilag, AstraZeneca and Sanofi-Aventis. T.P. has received education and research grants and honoraria for advisory boards and is a speaker for Astellas. Z.M. has received honoraria for advisory boards or is a speaker for Astellas, Amgen, GlaxoSmithKline, Jansen, Pierre Fabre, Roche and Sanofi. S.H. received has honoraria and travel support from Astellas, Sanofi, Jansen, Pfizer, Astra Zeneca, Pierre Fabre, Ipsen. A.R.-V. has received honoraria and travel support from Astellas, GlaxoSmithKline and Pfizer.

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Abbreviations: AAWS, antiandrogen withdrawal syndrome; CRPC, castration-resistant prostate cancer; AR, androgen receptor; OS, overall survival; PFS, progression-free survival; ECOG, Eastern Cooperative Group.

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8. Discussion

Our results support that the mortality of prostate cancer remains significant, challenging the traditional belief that prostate cancer is an indolent disease and is not an important cause of death in men. Our results also show for the first time that enzalutamide is associated with an antiandrogen withdrawal syndrome in a minority of patients and that its antitumour activity is significantly reduced when it is given to heavily pre-treated metastatic CRPC patients previously treated with abiraterone acetate. Furthermore, this indicates a phenomenon of cross-resistance between these 2 hormonal drugs targeting the AR signaling pathway. The incorporation of new hormonal agents such as abiraterone and enzalutamide has revolutionized the treatment of CRPC and significantly improved the OS of patients but has also raised the question of what is the ideal sequence for administering them. While cross-resistance between these agents when used in sequence has not been proven prospectively, our study suggests that the antitumour activity of enzalutamide is limited when given after abiraterone as compared to when given upfront. In the absence of randomized trials to determine the best sequence of treatment, our results advice against treating CRPC patients with abiraterone and enzalutamide sequentially.

In our first study, we showed that prostate cancer was the most frequent cause of death in the whole study population as well as in all the subgroups, regardless of age, stage of disease and treatment received (88). By the end of the follow up period, there had been 20.181 deaths (40.3%) among the 50.066 men with prostate cancer included in the study. Despite a relatively short median follow up of 3.5 years, almost half of these deaths (49.8%) were due to prostate cancer, representing the most common cause of death in the whole population. Age subgroups 65-74 and ≥75 included the 78% of patients of the

study, which reflects the fact that prostate cancer is most prevalent in older men. Unsurprisingly, the proportion of deaths rises with increasing age; however in all age groups prostate cancer remains the most frequent single cause of death. Even in the ≥75 years' subgroup, prostate cancer accounts for the 46.4% of deaths, despite the traditional belief that prostate cancer is a less aggressive disease when diagnosed at an advanced age and that elderly people with prostate cancer are more likely to die from other causes. This is a relevant finding, and should always be taken into consideration when making treatment decisions in elder men in order to avoid underestimating the lethal nature of prostate cancer. It is obviously very important not to over-treat frail elderly patients with severe comorbidities and a high likelihood of dying from other causes; but it is equally important to avoid to do harm by undertreating elderly patients with clinically detected prostate cancer just on the basis of an advanced age.

On the other hand, at younger ages the percentage of deaths due to prostate cancer as a proportion of all deaths increases (65.6% in the <65 years' subgroup compared to 46.4% in the ≥75 years subgroup) as other causes, most notably cardiovascular disease, become more infrequent. This supports the evidence that when prostate cancer is diagnosed at younger ages, it tends to have an aggressive behavior and will very likely ultimately be the cause of death of the patient. Prostate cancer staging at diagnosis obviously exerts a major effect on the proportion of deaths from prostate cancer itself, rising from 35.7% of all deaths in localized stage 1 disease to 74.3% for metastatic disease. However, even for patients who present with localized stage 1 disease, a situation where radical curative treatment options are available, prostate cancer is still the leading cause of death. Similarly, when analyzing the treatment received subgroup, even for patients treated with radical options such as prostatectomy or radiotherapy, prostate cancer was the most

frequent cause of death, especially in the radiotherapy group where 60.7% of deaths were due to prostate cancer. Finally, patients with advanced disease, represented by the stage 4 subgroup and the hormonal therapy-only subgroup, were also associated with a high proportion of prostate cancer deaths (74.3% and 56%, respectively), despite the fact that they were all treated in tertiary hospitals of the region of London in an era where new generation anticancer drugs and clinical trials were widely available. This striking high proportion of deaths due to prostate cancer in all subgroups regardless of age, stage and treatment, highlights therefore that prostate cancer has an enormous impact on the mortality of these individuals. Althought the fact that during the period of time when these patients were treated for prostate cancer, new generation anticancer drugs such as enzalutamide or abiraterone had not yet been approved, these were widely accessible through clinical trials in the region of London. Finally, surgery, radiotherapy and supportive care have all improved since the period of our study and therefore the percentage of men dying from prostate cancer in a contemporary cohort may be slightly lower.

Other studies have analyzed the cause of death in patients with prostate cancer in populations where PSA screening is more prevalent (91, 92). Unsurprisingly the CSM rate has been shown to be much lower than in our study. Lu-Yao *et al.* analyzed a population-based cohort study of 14.516 men aged 65 years or older diagnosed with localized prostate cancer (91). Among the whole study population, only 9.4% died due to prostate cancer compared to 20% in our study. Likewise, among the total number of deaths, only 20% were due to prostate cancer, compared to 49.8% in our study. Similar results were seen in another study looking at the CSM and other-cause mortality rates in 404.604 North American men with localized prostate cancer only (92). The 10-year CSM

rate was 6.1% and prostate cancer was the cause of death in only 16.6% of all deaths (compared to 49.8% in our study). It is likely that these significant differences are due to several factors. The most important factor is that our analysis also included men with locally advanced and metastatic disease and not only localized cases. Secondly, these 2 studies were carried out in USA centers where the uptake of PSA screening is high as compared to the UK or Europe in general and consequently, overdiagnosis of indolent prostate cancers might have diluted the real mortality rates of more aggressive and advanced prostate cancers.

In terms of mortality depending on staging, the comparison with data from the SEER program taken from USA cancer registries also shows some notable differences. Of note, the SEER stage distribution allocates only T1 and T2 disease as localized disease whereas the definition of localized disease used by TCR includes T3 disease. The SEER data on survival by stage at diagnosis for 2001-2007 shows a 5-year relative survival of 100% for those men with localized/regional disease and an overall 5-year relative survival of 99.4% (93). This is in contrast with overall relative survival of 68.7% seen in our study for stage 1 patients. Stage 1 disease in our cohort encompasses all local disease (T1-3) and is likely to be influenced by a significant proportion of symptomatic cases with higher local stage and grade. This contrasts with the SEER data where the group with localized disease is more dominated by asymptomatic men detected by PSA screening who often have low volume and low grade disease. Thus, the high proportion of deaths in stage 1 in our cohort likely reflects the presence of men with a more locally advanced disease for whom prostate cancer is the most significant pathology.

Despite the differences seen with these other similar analysis, our study is one of the few studies assessing prostate cancer mortality in a setting where uptake of PSA screening is low such as in the UK and most countries of Europe. Moreover, to our knowledge this is the largest study of its kind and benefits from the central collection and analysis of data. However, there are several limitations to the study. Firstly, the very same kind of study, the assessment of cause of death using death certificates, is both a quality and a potential weakness. Studies using death certificates to determine cause of death in prostate cancer have shown variable results and it remains uncertain as to how accurate this method is (94-96). However, the recent and relevant study by Godtman *et al.* used an independent cause of death committee and showed a 96% overall agreement with the initial cause of death using death certificates (95); this strengthens the reliability of our results. Secondly, the number of patients with stages 2 and 3 were relatively low in our study, and these data had to be combined due to the low numbers (1.168 men, 2.3%). The under-representation of stage 2 and 3 in our cohort may represent under-staging as relatively few patients may have had radiological or surgical staging. Finally, another important weakness of the study is the high prevalence of missing data: 46.3% of all men had no recorded treatment and 28.1% had an unknown stage. Nevertheless, whilst this may affect the exact estimates and interpretation relating to a specific stage and/or treatment, it does not affect the overall estimates of the proportion of men dying of prostate cancer which remains highly significant.

In our second study, we analyzed a cohort of patients treated with enzalutamide for metastatic CRPC in order to assess the antitumour activity of this hormone therapy in abiraterone pre-treated patients and to describe the kinetics of PSA following the discontinuation of enzalutamide and prior to starting further anticancer therapy. This

second study resulted in the publication of 2 articles, one for each sub-study: the Enzalutamide Cross-Resistance study and the Enzalutamide Withdrawal study (89, 90). In the Enzalutamide Cross-Resistance study, our most relevant discovery was that enzalutamide has a limited activity in the post-abiraterone patient population (89). We identified and analyzed 39 patients previously treated with abiraterone who went on to receive enzalutamide in the post-docetaxel setting. All the efficacy endpoints analyzed in our study were significantly poorer compared to the data reported in the AFFIRM study in abiraterone-naïve patients (11). Regarding PSA responses, the efficacy of enzalutamide in our cohort was significantly lower both in terms of PSA response rate and median treatment duration. The PSA response rate ≥50% was 12.8% (95% CI 5.6%-26.7%) compared to 54% in the AFFIRM study. Similarly, a confirmed PSA response rate of ≥90% was achieved in 2.6% (95% CI 0.4%-13.2%) of patients, and the median treatment duration was 2.9 months (95% CI 1.7-4.0), compared to 25% and 8.3 months, respectively in AFFIRM. Likewise, enzalutamide had a modest antitumour activity in terms of radiological response with a partial response rate of only 4.3% (95% CI 0.8-21) compared to 29% in AFFIRM. Our time-to-event efficacy endpoints were also significantly poorer with a median PFS of only 2.8 months (95% CI 2.0-3.6) (compared to 8.3 months, 95% CI 8.2-9.4) and a median time to PSA progression of 2.7 months (95% CI 2.5-3) (compared to 8.3 months, 95% CI 5.8-8.3). The cross-resistance between abiraterone and enzalutamide was also indicated by the observed correlation between prior response or not to abiraterone and the subsequent response or not to enzalutamide. Among patients who did not respond to upfront abiraterone in terms of PSA, 54.6% did not respond either to enzalutamide. This indicates that the molecular mechanisms of resistance to abiraterone might be partially shared by enzalutamide. Preclinical studies have actually proposed several possible mechanisms of resistance shared by abiraterone and

enzalutamide, including AR mutations (97, 98), constitutively active AR splice variants (99), AR activation by concomitant glucocorticoid use (100, 101) and activation of AR-independent signaling pathways such as the phosphatidylinositide 3-kinase (PI3K)-protein kinase B (AKT)-mammalian target of rapamycin (mTOR) pathway (102). Nevertheless, any mechanisms of cross-resistance between abiraterone and enzalutamide appear incomplete, since 1 patient whose disease had proved refractory to abiraterone had a 99.7% confirmed PSA decline and long-lasting response to enzalutamide. This indicates that different mechanisms of resistance are likely to exist in different patients, and perhaps even in the same patient, due to clonal heterogeneity as a result of disease clonal evolution induced by therapeutic selective pressure.

Following our study publication, several other retrospective studies have analyzed the antitumour activity of enzalutamide following progression on docetaxel and abiraterone and have confirmed the same poor results seen in our cohort (103-109). In 7 studies accounting for a total number of 341 patients with metastatic CRPC, PSA response rates ≥50% and ≥90% occurred in only 10-28.6% and 2.5-4.3% of patients respectively, in contrast to 54% and 25% in AFFIRM. Furthermore, in 2 of these studies between 36-55% of patients experienced continuous rising PSA as best PSA response to enzalutamide (104, 108). An ORR in measurable disease was seen in 2.9-11.8% of patients compared to 29% in the AFFIRM trial. All time-to-event endpoints were also significantly lower, with a median time to PSA progression, median PFS and median OS of 2.7-4.0 months, 2.7-4.9 months and 4.8-8.3 months respectively, as compared to 8.3 months, 8.3 months and 18.4 months in AFFIRM. Interestingly, among abiraterone-refractory patients, only 0-23.1% of them had a PSA responses ≥50% with enzalutamide, suggesting primary resistance and cross-resistance between the 2 agents (103, 105, 107). Conversely, among

abiraterone-sensitive patients, around 13.3-60% of them also had a PSA response rate ≥50% with enzalutamide, suggesting that there is a subset of patients who respond and benefit from both agents when given sequentially (103, 105, 107). Overall, these studies confirm and validate our findings that enzalutamide has a poor antitumoral activity in abiraterone pretreated patients.

In the Enzalutamide Withdrawal study, we showed for the first time that enzalutamide may have an antiandrogen withdrawal effect in a minority of patients (90). We initially identified 30 patients who progressed while receiving enzalutamide and for whom at least one PSA was available following enzalutamide discontinuation and prior to starting further therapy (results reported in the aforementioned article). A subsequent updated analysis increased the patient sample to 49 patients (results presented here). Only 1 patients (2%) had a confirmed PSA response ≥50% after stopping enzalutamide, meeting the pre-specified AAWS definition. In addition, 3 patients (6.1%) had a confirmed PSA response between 30-50% and another patient (2%) had an unconfirmed PSA response between 30-50%, which means that an AAWS ≥30% was seen in 10.2% of patients. This indicates that an AAWS is a possible yet rare event with enzalutamide. In total, a PSA reduction of any level in PSA was observed in 7 out of 49 patients after enzalutamide discontinuation (14.2%). The remaining 42 patients (85.7%) had a raised PSA after stopping enzalutamide. To our knowledge, this is the first study to examine PSA levels after enzalutamide cessation in patients with metastatic CRPC as well as the first one to show the existence of an AAWS with enzalutamide. Importantly, despite the withdrawal PSA response observed in our study, no symptomatic improvement or radiological responses were noted among patients experiencing a withdrawal effect. This puts into question the clinical relevance of the enzalutamide AAWS as a therapeutic maneuver as

has classically been debated with first-generation antiandrogens withdrawal syndrome. Median OS was longer, although not statistically significant, among patients with some enzalutamide AAWS (40.25 months, 95% CI 6.5-40.2) as compared to patients with rising PSA following enzalutamide discontinuation (15.5 months, 95% CI 8.1-24.7; p=0.307). However, the comparison of OS between both groups is severely limited by the small sample size. In the univariate analysis, only longer response to LHRH analogues was statistically significantly associated with higher probability of an enzalutamide AAWS (OR 1.03, 95% CI 1.01-1.06, $p<0.05$). Moreover, the patient that achieved a confirmed PSA response ≥50% had a prolonged duration of treatment with enzalutamide (21.4 months). Taken together, this information suggests that patients who respond longer to hormone therapy are more likely to experience an AAWS with enzalutamide. Interestingly, this is in keeping with what has previously been described with bicalutamide AAWS (21, 73).

Importantly, three recently published pre-clinical studies have shown for the first time that specific AR mutations can confer partial agonist activity to enzalutamide in *in vitro* and *in vivo* models of CRPC (110-112). Balbas and colleagues were the first to identify that prolonged treatment with enzalutamide in prostate cancer cell lines led to the spontaneous emergence of an AR novel specific mutation called F876L which converted enzalutamide into an AR agonist (110). Moreover, structural analysis of the mutated AR showed that F876L mutation affects the ligand-binding domain of the AR and is responsible for the switch of enzalutamide from AR antagonist to AR agonist. A similar study by Joseph *et al.* showed that F876L mutation was sufficient to convey acquired resistance not only to enzalutamide but also to another second-generation anti-androgen apalutamide in CRPC cell models (111). Finally another pre-clinical study showed that

F876L-bearing prostate cancer cells are not only resistant to enzalutamide but also dependent on the agonist effect of enzalutamide for cellular growth under androgen-deprivation conditions (112). In view of this addiction phenomenon to agonist enzalutamide, the authors hypothesize that patients bearing the F876L-mutated AR might clinically benefit from withdrawal of enzalutamide. Importantly, these studies provide a plausible molecular mechanism to explain the enzalutamide withdrawal syndrome seen in our study. Finally, these studies suggest that resistance to second-generation anti-androgens is still mediated by the AR and therefore progressive CRPC remains an AR-driven disease despite resistance to several hormone therapies.

There are several limitations to the Enzalutamide studies. Our second study is limited by the small patient population as well as the retrospective nature of the analysis. The modest efficacy seen with enzalutamide in our study, may have been influenced by the advanced disease stage of our population (ECOG PS 2 in 8.5% of patients enrolled in AFFIRM versus 35.8% in our cohort) resulting in reduced physical tolerance and worse outcomes. The likelihood of detecting an enzalutamide AAWS was limited by the fact that only 60% patients had a second confirmatory PSA checked following enzalutamide discontinuation. The remaining 40% patients started subsequent systemic therapies before a second PSA was measured. Also, enzalutamide withdrawal syndrome may have been underestimated by the relatively short period of PSA monitoring following enzalutamide withdrawal (median time 35 days, range 10-120). Whilst PSA responses after flutamide withdrawal usually occur within the first few days, bicalutamide withdrawal syndrome tends to appear 4-8 weeks after treatment discontinuation (21). It has been postulated that this different pattern of PSA withdrawal response is probably due to the long half-life of bicalutamide (t1/2= 5.9 days) (113) compared to flutamide (t1/2= 6 hours) (114).

Enzalutamide has a similar half-life than bicalutamide (t1/2= 5.8 days) (115) which means that in order to assess a potential AAWS with enzalutamide, PSA levels should ideally be monitored during 8 weeks following treatment discontinuation. In our study however, due to the fact that 60% of patients started further systemic therapies, PSA levels were only monitored for a median time of 35 days (5 weeks). Unfortunatelly, we could not sequence the AR gen in our patient presenting an AAWS with enzalutamide in order to test for potential AR mutations such as the F876l. Finally, since AAWS has been described more frequently at early stages of metastatic prostate cancer, the possibility of an enzalutamide AAWS in our study may have been reduced due to the heavily pre-treated status of the study population.

Despite these limitations, our second study showed for the first time that enzalutamide is associated with an antiandrogen withdrawal syndrome in a minority of patients and that its antitumour activity is significantly reduced when it is given to heavily pre-treated metastatic CRPC patients previously treated with abiraterone acetate. With the successful development and approval of cabazitaxel, abiraterone and enzalutamide, oncologists are facing the difficult task of selecting the optimal sequence for patients with CRPC progressing to docetaxel. To date, no clinical or biological factors have been shown to predict response to these agents and treatment choices are based on availability, patient/clinician preferences and performance status or comorbidities. Overall, enzalutamide remained a safe and relatively well tolerated treatment in our study patient population. Therefore, in the absence of better clinical or biological predictive factors of treatment response, the activity of enzalutamide after abiraterone may represent a valid treatment option for advanced prostate cancer patients who remain in a good performance status after progressing to docetaxel and abiraterone, especially is they responded to

abiraterone. However, for patients who presented with primary resistance as best response to abiraterone, a sequential treatment with enzalutamide should be ill-advised given the high probability of cross-resistance and non-hormonal agents should be favoured. Finally, despite the infrequent prevalence of a withdrawal syndrome with enzalutamide seen in our study and its questionable clinical meaningfulness, its simple recognition as a new entity is very relevant as it may improve our understanding of the resistance mechanisms to hormone therapy and may help in the development of the next generation AR targeted agents.

In conclusion, enzalutamide has modest antitumour activity in advanced-stage CRPC patients when used after abiraterone. In a small subset of patients, enzalutamide can be associated with a withdrawal response. Larger prospective studies are now needed to confirm the existence and clinical relevance of an enzalutamide withdrawal syndrome and to provide further data regarding optimal hormone treatment sequencing.

9. Conclusions

Prostate Cancer Mortality Study (First Article):

- In our population-based epidemiological study, prostate cancer was the most frequent cause of death in a UK cohort of 50.066 men with prostate cancer, regardless of age, stage of disease and treatment received.

- Despite a relatively short median follow up of 3.5 years, almost half of the deaths (49.8%) were due to prostate cancer, representing the most common cause of death in the whole population.

- Prostate cancer is also the most common cause of death in patients treated with radical treatment options (prostatectomy, radiotherapy) as well as in patients with stage 4 disease, despite the fact that all patients were all treated in tertiary hospitals of the region of London in an era where new generation anticancer drugs and clinical trials were available.

- The mortality of prostate cancer remains significant. This challenges the traditional belief that prostate cancer is an indolent disease and is not an important cause of death in men.

Enzalutamide Cross-Resistance Study (Second Article):

- We showed for the first time that the antitumour activity of enzalutamide is significantly reduced when it is given to metastatic CRPC patients previously treated with abiraterone.

- All the efficacy endpoints analyzed in our cohort were significantly poorer compared to the data reported in the AFFIRM study in abiraterone-naïve patients, including PSA response rates, radiological response rates and survival

- There was a high correlation between prior response or not to abiraterone and the subsequent response or not to enzalutamide. This indicates the existence of cross-resistance between these 2 agents and that the molecular mechanisms of resistance to abiraterone might be partially shared by enzalutamide.

Enzalutamide Withdrawal Study (Third Article):

- We showed for the first time that enzalutamide may have an antiandrogen withdrawal effect in a minority of patients (2%).

- No symptomatic improvement or radiological responses were noted among patients experiencing a withdrawal effect. This puts into question the clinical relevance of the enzalutamide AAWS as a therapeutic maneuver.

- On univariate analysis, longer response to LHRH analogues was statistically significantly associated with higher probability of an enzalutamide AAWS ($p<0.05$).

- Larger prospective studies are now needed to confirm the existence and clinical relevance of an enzalutamide withdrawal syndrome and to provide further data regarding optimal hormone treatment sequencing.

10. Resumen en castellano

Cáncer de próstata: mortalidad e impacto de los inhibidores del receptor de andrógenos de segunda generación

10.1 Introducción:

El cáncer de próstata es el segundo cáncer diagnosticado con mayor frecuencia en todo el mundo y la quinta causa de muerte por cáncer en varones, con una incidencia estimada de 1.1 millones casos nuevos y 307.000 muertes en 2012 (1). En los últimos 20-30 años, ha habido un gran aumento en la incidencia de cáncer de próstata en la Unión Europea y en todo el mundo, probablemente debido al uso generalizado de la resección transuretral de la próstata y a la detección precoz del antígeno prostático específico (PSA) (4). El cáncer de próstata es una enfermedad heterogénea que va desde casos localizados indolentes hasta casos agresivos e indiferenciados con tendencia a propagarse y convertirse en metastásicos e incurables. La existencia de una pequeña proporción de pacientes afectos de un tipo de cáncer de próstata más indolente ha llevado a la creencia generalizada de que el cáncer de próstata tiene una baja letalidad y que la mayoría de los pacientes con cáncer de próstata morirán con su enfermedad, más que debido a ella. El uso generalizado de la detección oportunista del PSA en pacientes asintomáticos en algunos países, como en los Estados Unidos de América, podría explicar parcialmente esta creencia. Se sabe que la detección precoz de PSA tiende a sobrediagnosticar una proporción significativa de tumores indolentes en estadios más tempranos, algunos de los cuales podrían haber permanecido siempre latentes sin nunca causar síntomas o la muerte del paciente (5). En consecuencia, el sobrediagnóstico de cánceres de próstata indolentes podría haber diluido la tasa de mortalidad real de las variantes de cáncer de próstata más

agresivos y avanzados. Se necesitan por tanto urgentemente estudios epidemiológicos que analicen la mortalidad real del cáncer de próstata y las causas de muerte en los países donde la detección precoz de PSA no es corriente y así proporcionar una imagen real del impacto del cáncer de próstata en la mortalidad.

La terapia de deprivación androgénica (TDA) con análogos de la hormona liberadora de gonadotrofina (HLG) o mediante orquidectomía bilateral es el principal tratamiento de primera línea en pacientes con cáncer de próstata metastásico. Sin embargo, tras una respuesta de 18 a 24 meses de media, la mayoría de los pacientes finalmente experimentan progresión de la enfermedad entrando en la fase de resistencia a la castración (CPRC) (8, 9). Esta fase de la enfermedad se catalogó en el pasado como hormono-refractaria o resistente a tratamiento hormonal. Sin embargo, estos términos son inexactos y han caído en desuso desde que estudios recientes demostraron que incluso en esta fase, la progresión de la enfermedad sigue estando principalmente impulsada por la señalización del receptor de andrógenos (RA). Consecuentemente, en los últimos años se han probado con éxito varios nuevos inhibidores de la vía del RA de nueva generación en pacientes con CPRC metastásico confirmando la hipótesis de que la progresión del cáncer de próstata sigue dependiendo en todo momento de la estimulación androgénica. Es el caso del acetato de abiraterona, un potente inhibidor del CYP17, y de enzalutamida, un antiandrógeno de segunda generación. Ambos han demostrado prolongar la supervivencia global (SG) y mejorar la calidad de vida de pacientes con CPRC metastásicos previamente tratados o no con docetaxel (10-13). Sin embargo, a pesar de una buena respuesta inicial a estos nuevos fármacos, la mayoría de los pacientes finalmente experimentará resistencia a dichos agentes y progresión de la enfermedad. Los ensayos clínicos aleatorizados que condujeron a la aprobación tanto de enzalutamida como de abiraterona, no permitían la

inclusión de pacientes que hubiesen sido previamente tratados con el otro fármaco, respectivamente. Por lo tanto, aunque la eficacia individual de abiraterona y enzalutamida en el cáncer de próstata está bien demostrada, el beneficio terapéutico de administrar de forma secuencial estos dos inhibidores de la vía del RA no está claro. De hecho, se han publicado varios estudios retrospectivos preliminares que sugieren que abiraterona tiene una actividad antitumoral reducida cuando se administra en paciente previamente tratados con enzalutamida, lo que indica la existencia de resistencia cruzada entre estos dos agentes (19, 20). Por el contrario, la actividad antitumoral de enzalutamida en pacientes previamente tratados con abiraterona es aún desconocida, aunque se puede hipotetizar que un fenómeno de resistencia cruzada podría ocurrir de manera similar.

Los mecanismos moleculares detrás de la resistencia cruzada entre inhibidores de la vía del RA siguen siendo poco conocidos. La experiencia adquirida previamente con los inhibidores del RA de primera generación, como la bicalutamida, podría proporcionarnos algunas hipótesis para comprender mejor dichos mecanismos. Antes del advenimiento de los inhibidores de segunda generación, el uso de bicalutamida como tratamiento hormonal de segunda línea en pacientes con cáncer de próstata metastásico que habían progresado a la TDA era muy común. En aquellos pacientes que posteriormente volvían a experimentar progresión pese a bicalutamide, la retirada de dicho fármaco se asociaba a una reducción paradójica del PSA en un 15-30% de pacientes (21, 22) en lo que era conocido como el síndrome de retirada del antiandrógeno (SRAA). A pesar de que se ha descrito un SRAA con casi todos los antiandrógenos de primera generación como la bicalutamida o la flutamida, no hay ninguna evidencia de SRAA con antiandrógenos de segunda generación como la enzalutamida. A diferencia de la bicalutamida, enzalutamida no demostró tener ningún efecto agonista del RA en los estudios preclínicos (24). Por lo

tanto, dado que enzalutamida es un antagonista puro del RA, se cree que no puede asociarse a un SRAA. Sin embargo, la existencia o no de un SRAA con enzalutamida nunca ha sido investigada en el contexto clínico.

10.2 Hipótesis y objetivos

10.2.1 Hipótesis

-La primera hipótesis de este estudio es que la mortalidad del cáncer de próstata sigue siendo significativa a pesar de la incorporación de fármacos de nueva generación, especialmente en un entorno donde la detección precoz de PSA es baja, como en el Reino Unido. Esto desafía la creencia tradicional de que el cáncer de próstata no es una causa importante de muerte en varones.

-La segunda hipótesis es que la actividad antitumoral de enzalutamida en pacientes con cáncer de próstata resistente a la castración metastásico previamente tratados con abiraterona es significativamente inferior en comparación con cuando se administra enzalutamida a pacientes que no han recibido abiraterona previamente. Esta actividad antitumoral reducida de enzalutamida en pacientes tratados previamente con abiraterona indicaría la existencia de resistencia cruzada entre estos dos fármacos hormonales que actúan sobre la vía de señalización del RA.

-La tercera y última hipótesis es que el tratamiento mantenido con enzalutamida puede conducir al desarrollo de un efecto agonista parcial del RA ilustrado por la aparición de un SRAA al retirar la enzalutamida. Los mecanismos moleculares subyacentes de dicho SRAA podrían proporcionar información sobre los mecanismos potenciales de resistencia a la enzalutamida.

10.2.2 Objetivos

-Investigar la mortalidad y las causas de muerte en varones con cáncer de próstata diagnosticado en Londres (Reino Unido), donde el uso de la detección precoz de PSA en varones asintomáticos es bajo. Examinar la relación entre la causa de muerte y las características del paciente en el momento del diagnóstico, incluyendo edad, estadio del cáncer y tratamiento recibido. Calcular para cada factor, la proporción de muertes debidas a cáncer de próstata, y la incidencia acumulada de muerte por cáncer de próstata.

-Analizar la actividad antitumoral de enzalutamida en términos de respuesta bioquímica por PSA, respuesta radiológica y supervivencia en pacientes con cáncer de próstata metastásico resistente a la castración previamente tratados con abiraterona. Comparar dichos resultados con los datos publicados sobre la actividad de enzalutamida cuando se administra en pacientes no previamente tratados con abiraterona.

-Examinar los niveles de PSA tras la interrupción de enzalutamida en pacientes con cáncer de próstata metastásico resistente a la castración, con el fin de evaluar si existe un síndrome de retirada del antiandrógeno con enzalutamida. Correlacionar el PSA post-retirada con supervivencia. Correlacionar los factores clínicos y terapéuticos de los pacientes que se asocian con un SRAA potencial con enzalutamida.

10.3 Resultados (Artículos)

10.3.1 Causas de muerte en varones con cáncer de próstata: análisis de mortalidad en 50.000 varones del Thames Cancer Registry

Este estudio examinó las causas de muerte en 50.066 varones con cáncer de próstata diagnosticados entre 1997 y 2006 y recogidas en el Thames Cancer Registry, un registro

epidemiológico poblacional de la región de Londres y el sudeste del Reino Unido. Para ello, se recogieron las causas subyacentes de muerte a través de los certificados de defunción de dichos pacientes. Durante ese periodo, la detección precoz del PSA era baja en el Reino Unido. Examinamos la relación entre la causa de muerte y las características del paciente en el momento del diagnóstico, incluyendo edad, estadio del cáncer y tratamiento oncológico recibido dentro de los seis meses posteriores al diagnóstico.

Resultados: Se detectaron 20.181 muertes durante el período del estudio. El 49.8% de estas muertes se debieron a cáncer de próstata, el 17.8% a enfermedades cardiovasculares, el 11.6% a otros cánceres y el 20.7% a otras causas. Independientemente de la edad, el estadio del cáncer o el tratamiento oncológico recibido dentro de los seis meses posteriores al diagnóstico, el cáncer de próstata fue una causa importante de muerte oscilando entre el 31% y el 74% del total de muertes en los diferentes subgrupos.

Conclusión: la mortalidad debida al cáncer de próstata en los varones incluidos en nuestro estudio fue significativa, independientemente de los factores basales. En un entorno epidemiológico donde la detección precoz del PSA es bajo, como es el caso del Reino Unido, nuestros resultados desafían la creencia de que el cáncer de próstata no es una causa importante de muerte.

10.3.2 La actividad antitumoral de enzalutamida en pacientes con cáncer de próstata metastásico resistente a la castración previamente tratados con docetaxel y abiraterona

Este estudio evaluó la actividad antitumoral de enzalutamida en pacientes con cáncer de próstata resistente a la castración metastásico previamente tratados con abiraterona.

Identificamos retrospectivamente pacientes tratados con docetaxel y abiraterona previamente a la administración de enzalutamida. La actividad antitumoral de enzalutamida fue analizada mediante la determinación de la respuesta bioquímica por PSA, de la respuesta radiológica y de la supervivencia asociada a enzalutamida.

Resultados: se identificaron 39 pacientes con cáncer de próstata metastásico resistentes a la castración. En total, 16 pacientes (41%) tuvieron una respuesta bioquímica de PSA confirmada superior o igual al 30%. Una respuesta bioquímica de PSA confirmada superior o igual al 50% y al 90% fue observada en 5 pacientes (12.8%) y en 1 paciente (2.5%), respectivamente. De los 15 pacientes que respondieron a abiraterona, dos (13.3%) también tuvieron una respuesta bioquímica de PSA confirmada superior o igual al 50% con el subsiguiente tratamiento con enzalutamida. Entre los 22 pacientes refractarios a abiraterona, dos (9%) lograron una respuesta bioquímica de PSA confirmada superior o igual al 50% de PSA en enzalutamida.

Conclusión: Estos datos de actividad antitumoral de enzalutamida son significativamente inferiores a los publicados en los ensayos aleatorizados de enzalutamida en pacientes no previamente tratados con abiraterona. Los datos de nuestro estudio sugieren una actividad antitumoral reducida de enzalutamida en los pacientes previamente tratados con abiraterona, indicando una posible resistencia cruzada entre dichos fármacos.

10.3.3 Existe el síndrome de retirada del antiandrógenos con enzalutamida?

Este estudio analizó los niveles de PSA tras la interrupción de enzalutamida en pacientes con cáncer de próstata metastásico resistente a la castración, con el fin de evaluar si existe un síndrome de retirada del antiandrógeno con enzalutamida. Para ello identificamos

retrospectivamente 30 pacientes con cáncer de próstata metastásico tratados con enzalutamida. Sólo se incluyeron aquellos pacientes para los cuales se disponía de como mínimo una determinación de PSA tras la retirada de enzalutamida. El SRAA se definió como una disminución del PSA ≥50% en comparación con la última determinación de PSA durante el tratamiento, confirmado con una segunda disminución 3 semanas más tarde. Se realizó un análisis de regresión logística univariante para analizar la relación entre las características clínicas y terapéuticas de los pacientes y la aparición del SRAA.

Resultados: Enzalutamida fue retirada debido a progresión clínica en el 73.3% de los pacientes, progresión bioquímica de PSA en el 90% y/o progresión radiológica en el 60%. Los niveles de PSA después de la retirada de enzalutamida se monitorizaron durante un tiempo medio de 35 días (rango 10-120). Únicamente un paciente (3.3%) presentó una reducción confirmada del PSA ≥50% después de la interrupción de enzalutamida. Un paciente (3.3%) tuvo una reducción confirmada del PSA entre el 30-50% y otro paciente (3.3%) tuvo una respuesta no confirmada PSA entre 30-50%.

Conclusiones: Nuestro estudio proporciona la primera evidencia de que enzalutamida puede tener un SRAA en una minoría de pacientes con CPRC metastásico. Se deberán realizar más estudios para confirmar la existencia de un SRAA con enzalutamida y para evaluar su relevancia clínica en el tratamiento del cáncer de próstata.

10.4 Discusión

Nuestros resultados muestran que la mortalidad del cáncer de próstata sigue siendo significativa, desafiando la creencia tradicional de que el cáncer de próstata es una enfermedad mayoritariamente indolente y no una causa importante de muerte en los

varones. Nuestros resultados también muestran por primera vez que enzalutamida está asociada con un síndrome de retirada del antiandrógeno en una minoría de pacientes y que su actividad antitumoral se reduce significativamente cuando se administra en pacientes con CPRC metastásicos previamente tratados con acetato de abiraterona. Esto indica que existe un fenómeno de resistencia cruzada entre estos 2 fármacos hormonales dirigidos a la vía de señalización del receptor de andrógenos.

En nuestro primer estudio, demostramos que el cáncer de próstata fue la causa de muerte más frecuente en toda la población estudiada, así como en todos los subgrupos, independientemente de la edad, estadio de la enfermedad y tratamiento recibidos (88). A pesar de una mediana de seguimiento relativamente corta (3.5 años), casi la mitad de las muertes (49.8%) se debieron al cáncer de próstata, siendo la causa de muerte más común en toda la población. Como era de esperar, la proporción de muertes aumenta con el aumento de la edad; sin embargo, en todos los grupos de edad, el cáncer de próstata sigue siendo la causa de muerte más frecuente. En el subgrupo de ≥75 años, el cáncer de próstata representa el 46.4% de las muertes, a pesar de la creencia tradicional de que el cáncer de próstata es una enfermedad menos agresiva cuando se diagnostica a una edad avanzada y que las personas mayores con cáncer de próstata fallecerán probablemente por otras causas. Este es un hallazgo relevante, y siempre debería tenerse en cuenta al tomar decisiones terapéuticas en varones ancianos a fin de evitar subestimar la naturaleza letal del cáncer de próstata. Obviamente, es muy importante no sobre-tratar a los pacientes ancianos frágiles con comorbilidades importantes y una alta probabilidad de morir por otras causas. Sin embargo, es igualmente relevante evitar el daño causado por tratar de forma subóptima a los pacientes ancianos con cáncer de próstata sólo en base a una edad avanzada.

Por otra parte, en la franja de edades más jóvenes, el porcentaje de muertes por cáncer de próstata como proporción de todas las muertes fue mayor (65.6% en el subgrupo <65 años, en comparación con el 46.4% en el subgrupo de ≥75 años). Esto apoya la evidencia de que cuando el cáncer de próstata se diagnostica a edades más tempranas, tiende a tener un comportamiento agresivo y muy probablemente ocasionará en última instancia la muerte del paciente. Evidentemente, el estadio del cáncer de próstata al diagnóstico ejerce un efecto importante en la proporción de muertes debidas a cáncer de próstata: el 35.7% de todas las muertes en pacientes con enfermedad localizada (estadio 1) fueron debidas a cáncer de próstata frente al 74.3% en pacientes con enfermedad metastásica. Sin embargo, incluso para los pacientes con enfermedad localizada, una situación donde existen las opciones de tratamiento curativo radical, el cáncer de próstata sigue siendo la principal causa de muerte. Por último, los pacientes con enfermedad avanzada, representada por el subgrupo de estadio 4 y el subgrupo que sólo recibió terapia hormonal, también se asociaron con una alta proporción de muertes por cáncer de próstata (74.3% y 56%, respectivamente), a pesar de que todos ellos fueron tratados en hospitales de tercer nivel de la región de Londres en una era donde los fármacos de nueva generación contra el cáncer y los ensayos clínicos eran ampliamente disponibles. Esta sorprendente alta proporción de muertes debidas al cáncer de próstata en todos los subgrupos estudiados, implica por tanto que el cáncer de próstata tiene un enorme impacto en la mortalidad de estos pacientes.

Otros estudios similares han analizado la causa de la muerte en pacientes con cáncer de próstata en poblaciones americanas, obteniendo una tasa de mortalidad por cáncer de próstata menor que en nuestro estudio (91, 92). En estos estudios, únicamente el 6.1% y

el 9,4% de los pacientes respectivamente murieron debido al cáncer de próstata en comparación con el 20% en nuestro estudio. Del mismo modo, de entre el total de fallecimientos, sólo el 16.6%-20% se debieron al cáncer de próstata, en comparación con el 49,8% de nuestro estudio. Estas diferencias se deben a varios factores. El factor más significativo es que nuestro análisis también incluyó pacientes con enfermedad localmente avanzada y metastásica y no sólo casos localizados. En segundo lugar, estos dos estudios se llevaron a cabo en centros norteamericanos donde la detección precoz de PSA es alta en comparación con el Reino Unido o Europa. En consecuencia, el sobrediagnóstico de casos de cáncer de próstata más indolentes podría haber diluido la tasa de mortalidad real de los casos de cáncer de próstata más agresivos y avanzados.

A pesar de las diferencias observadas con estudios similares, nuestro estudio es uno de los pocos que ha evaluado la mortalidad por cáncer de próstata en un entorno en el que la detección precoz de PSA es baja, como en Europa. Además, según nuestro conocimiento, éste es el estudio de mortalidad en cáncer de próstata más amplio publicado hasta la fecha y se caracteriza por la fortaleza de tener una recopilación y análisis centrales de los datos. Sin embargo, el estudio tiene varias limitaciones. En primer lugar, el tipo de estudio en sí, la evaluación de la causa de muerte utilizando certificados de defunción, es tanto una calidad como una debilidad potencial. Los estudios que utilizan certificados de defunción para determinar la causa de la muerte en cáncer de próstata han mostrado resultados variables y sigue siendo controvertido cuán de fiable es realmente este método (94-96). En segundo lugar, el número de pacientes con estadios 2 y 3 fue relativamente bajo en nuestro estudio (2.3%). Esta representación subóptima de los estadios 2 y 3 en nuestra cohorte podría deberse a una estadificación subóptima ya que relativamente pocos pacientes deben haber sido estadificados de forma radiológica o quirúrgica. Por último,

otra limitación importante del estudio es la alta frecuencia de datos no disponibles: el 46.3% de los pacientes no tenían tratamiento registrado y el 28.1% tenían un estadio desconocido. Aún y así, aunque esto último podría haber afectado las estimaciones exactas y la interpretación relativa a un estadio y/o tratamiento específicos, las estimaciones globales de la proporción de varones que mueren por cáncer de próstata no se ven afectadas y siguen siendo altamente significativas.

En nuestro segundo estudio, analizamos una cohorte de pacientes tratados con enzalutamida con el fin de evaluar la actividad antitumoral de esta terapia hormonal en pacientes con CPRC metastásicos previamente tratados con abiraterona y para describir la cinética de PSA después de la retirada de enzalutamida y antes de iniciar otra terapia antitumoral. Este segundo estudio resultó en la publicación de 2 artículos, uno para cada subestudio: el estudio de Resistencia Cruzada con Enzalutamida y el estudio de Retirada de Enzalutamida (89, 90). En el estudio de Resistencia Cruzada con Enzalutamida, nuestro descubrimiento más relevante fue que enzalutamida tiene una actividad limitada en los pacientes pretratados con abiraterona (89). Identificamos 39 pacientes con CPRC metastásico previamente tratados con abiraterona y docetaxel y que posteriormente recibieron enzalutamida. Todos los criterios de valoración de la eficacia analizados en nuestro estudio fueron significativamente más pobres en comparación con los datos reportados en el estudio AFFIRM en pacientes no pretratados con abiraterona (11). La tasa de respuesta de PSA ≥50% fue del 12.8% (intérvalo de confianza [IC] del 95% 5.6%-26.7%) en comparación con el 54% en el estudio AFFIRM. La duración mediana del tratamiento fue de 2.9 meses (IC 95% 1.7-4.0), frente a 8.3 meses en AFFIRM. Del mismo modo, enzalutamida tuvo una modesta actividad antitumoral en términos de respuesta radiológica con una tasa de respuesta parcial de sólo 4.3% (IC 95% 0.8-21) en

comparación con 29% en AFFIRM. La supervivencia libre de progresión (SLP) mediana fue de sólo 2.8 meses (IC del 95%: 2.0-3.6) comparado con 8.3 meses (IC 95% 8.2-9.4); y la mediana de tiempo hasta la progresión del PSA fue de 2.7 meses (IC 95% 2.5-3.0) en comparación con 8.3 meses (IC 95% 5.8-8.3). La resistencia cruzada entre abiraterona y enzalutamida también fue sugerida por la correlación observada entre la presencia o no de respuesta previa a abiraterona y la respuesta subsiguiente a enzalutamida. Entre los pacientes que no respondieron a abiraterona en términos de PSA, el 54.6% tampoco respondió a enzalutamida. Esto indica que los mecanismos moleculares de resistencia a la abiraterona podrían ser parcialmente compartidos por enzalutamida.

Después de la publicación de nuestro estudio, otros grupos han analizado también de forma retrospectiva la actividad antitumoral de enzalutamida después de la progresión a docetaxel y abiraterona y han confirmado los mismos resultados desfavorables observados en nuestra cohorte (103-109). En 7 estudios con un total de 341 pacientes con CPRC metastásico, las tasas de respuesta de PSA ≥50% ocurrieron en sólo 10-28.6% de los pacientes, en contraste con el 54% en AFFIRM. Además, en 2 de estos estudios, entre 36-55% de los pacientes experimentaron un aumento progresivo del PSA como mejor respuesta a enzalutamida (104, 108). La tasa de respuestas radiológicas fue sólo de 2.9-11.8% en comparación con el 29% en el ensayo AFFIRM. Entre los pacientes refractarios a abiraterona, sólo el 0-23.1% presentaron una respuesta de PSA ≥50% con enzalutamida, lo que sugiere resistencia primaria y resistencia cruzada entre los 2 agentes (103, 105, 107). Por el contrario, entre los pacientes sensibles a abiraterona, alrededor del 13.3-60% también tuvieron una respuesta de PSA ≥50% con enzalutamida, lo que sugiere que hay un subgrupo de pacientes que responden y se benefician de ambos agentes cuando se administran secuencialmente (103, 105, 107). Tomados en conjunto, estos estudios

confirman y validan nuestros hallazgos de que enzalutamida tiene una reducida actividad antitumoral en pacientes previamente tratados con abiraterona.

En el estudio de Retirada de Enzalutamida, demostramos por primera vez que enzalutamida puede tener un efecto de retirada del antiandrógeno en una minoría de pacientes (90). Inicialmente identificamos 30 pacientes que progresaron mientras recibían enzalutamida y para los cuales había al menos una determinación de PSA disponible tras la retirada de enzalutamida y antes de iniciar otra terapia (resultados publicados en el artículo mencionado previamente). Un análisis posterior actualizado aumentó la muestra disponible a 49 pacientes (resultados aquí reportados). Sólo 1 paciente (2%) presentó una reducción de PSA confirmada ≥50% después de retirar enzalutamida cumpliendo con la definición pre-establecida de SRAA. Además, otros 3 pacientes (6,1%) tuvieron una reducción de PSA confirmada entre 30-50% y otro paciente (2%) presentó una respuesta de PSA no confirmada entre 30-50%, lo que significa que se observó un SRAA de ≥30% en el 10.2% de pacientes. En total, se observó una reducción del PSA de cualquier nivel en 7 de 49 pacientes tras de la interrupción de enzalutamida (14.2%). Los restantes 42 pacientes (85.7%) presentaron un aumento progresivo de PSA. Nuestro estudio indica por tanto que el SRAA es un evento posible aunque raro con enzalutamida. En nuestro conocimiento, éste es el primer estudio publicado hasta la fecha que haya examinado los niveles de PSA después de la retirada de enzalutamida, así como el primero en demostrar la existencia de un SRAA con enzalutamida. Sin embargo, la ausencia de mejoría sintomática o de respuestas radiológicas en paralelo con el SRAA, pone en tela de juicio la relevancia clínica de este síndrome como maniobra terapéutica, como ya se ha debatido clásicamente con el síndrome de retirada de los antiandrógenos de primera generación. En el análisis univariante, únicamente una respuesta más larga a los análogos de HLG se

asoció de forma estadísticamente significativa con una mayor probabilidad de presentar un SRAA con enzalutamida (OR 1.03, IC 95% 1.01-1.06, $p<0.05$). Esto sugiere que los pacientes que responden más tiempo a la terapia hormonal son más propensos a experimentar un SRAA con enzalutamida, hecho que ya ha sido previamente descrito anteriormente con el SRAA de bicalutamida (21, 73). Es importante destacar que tres estudios preclínicos publicados recientemente han demostrado por primera vez que mutaciones específicas del RA pueden conferir actividad agonista parcial a enzalutamida en modelos *in vitro* e *in vivo* de CPRC (110-112) y que estos pacientes podrían beneficiarse de la retirada de enzalutamida. Estos estudios proporcionan por tanto un mecanismo molecular plausible para explicar el síndrome de retirada de enzalutamida observado en nuestro estudio.

Los 2 estudios de Enzalutamida tienen varias limitaciones. En primer lugar, ambos estudios están limitados por la pequeña muestra de pacientes incluidos, así como por la naturaleza retrospectiva del análisis. La reducida eficacia antitumoral observada con enzalutamida en nuestro estudio podría haber sido influenciada por el hecho que la mayoría de los pacientes incluidos se hallaban en una etapa muy avanzada de la enfermedad donde la respuesta a cualquier tratamiento es menor. La probabilidad de detectar un SRAA con enzalutamida se vio limitada por el hecho de que sólo el 60% de los pacientes tuvieron una segunda determinación de PSA tras la retirada de enzalutamida. Teniendo en cuenta que el SRAA con bicalutamida puede tardar en aparecer entre 4-8 semanas tras la retirada, nuestros datos están limitados por el hecho que el tiempo medio de monitorización de PSA en nuestro estudio fue de sólo 5 semanas tras la retirada de enzalutamida. Esto podría infravalorar la prevalencia del SRAA. A pesar de estas limitaciones, nuestro segundo estudio demostró por primera vez que enzalutamida se

asocia con un síndrome de retirada del antiandrógeno en una minoría de pacientes y que su actividad antitumoral se reduce significativamente cuando se administra a pacientes previamente tratados con acetato de abiraterona. Ambos descubrimientos son relevantes ya que contribuyen a incrementar nuestro conocimiento sobre los mecanismos de resistencia a terapia hormonal y esto podría ser de gran ayuda para el desarrollo futuro de los inhibidores del receptor de andrógenos de nueva generación.

10.5 Conclusiones

Estudio de mortalidad en cáncer de próstata (Primer Artículo):

- En nuestro estudio epidemiológico, el cáncer de próstata fue la causa de muerte más frecuente en una cohorte del Reino Unido de 50.066 varones con cáncer de próstata, independientemente de la edad, el estadio de la enfermedad y el tratamiento recibido.

- A pesar de un seguimiento mediano relativamente corto (3.5 años), casi la mitad de las muertes (49.8%) se debieron al cáncer de próstata.

- El cáncer de próstata es también la causa más común de muerte en pacientes tratados con opciones de tratamiento radical (prostatectomía, radioterapia), así como en pacientes con enfermedad en estadio 4, a pesar de que todos los pacientes fueron tratados en hospitales de tercer nivel de la región de Londres en una era en que los fármacos nueva generación contra el cáncer y los ensayos clínicos estaban ampliamente disponibles.

- La mortalidad por cáncer de próstata sigue siendo significativa. Esto desafía la creencia tradicional de que el cáncer de próstata es una enfermedad indolente y no es una causa importante de muerte en varones.

Estudio de Resistencia Cruzada con Enzalutamida (Segundo Artículo):

- Demostramos por primera vez que la actividad antitumoral de enzalutamida se reduce significativamente cuando se administra a pacientes con CPRC metastásico previamente tratados con acetato de abiraterona.

-Todos los criterios de valoración de la eficacia analizados en nuestra cohorte fueron significativamente más pobres en comparación con los datos reportados en el estudio AFFIRM en pacientes no previamente tratados con abiraterona, incluido la tasa de respuesta por PSA, la tasa de respuesta radiológica y la supervivencia.

- Hubo una alta correlación entre la presencia o no de respuesta previa a la abiraterona y la respuesta subsiguiente a enzalutamida. Esto es indicativo de que existe resistencia cruzada entre estos 2 agentes y que los mecanismos moleculares de resistencia a abiraterona podrían ser parcialmente compartidos por enzalutamida.

Estudio de Retirada de Enzalutamida (Tercer Artículo):

- Demostramos por primera vez que enzalutamida puede tener un efecto de retirada en una minoría de pacientes (2%).

- No se observaron mejorías sintomáticas o respuestas radiológicas entre los pacientes que experimentaron un efecto de retirada. Esto pone en duda la relevancia clínica del SRAA de enzalutamida como maniobra terapéutica.

- En el análisis univariado, una respuesta de mayor duración a los análogos de HLG se asoció de forma estadísticamente significativa con una mayor probabilidad de presentar un SRAA con enzalutamida ($p<0.05$).

- Se necesitan estudios prospectivos para confirmar la existencia y la relevancia clínica del síndrome de retirada con enzalutamida y para proporcionar más datos sobre la secuencia óptima de tratamiento hormonal en cáncer de próstata.

11. Abbreviations list

AAWS: antiandrogen withdrawal syndrome

ADT: androgen deprivation therapy

AE: adverse event

AKT: protein kinase B

ALT: alanine aminotransferase

AR: androgen receptor

AST: aspartate aminotransferase

CI: confidence interval

CPRC: cáncer de próstata resistente a la castración

CR: complete response

CRPC: castration-resistant prostate cancer

CSM: cancer specific mortality

CT: computed tomography

CTCAE: Common Terminology Criteria for Adverse Events

CYP17: cytochrome P450c17

DHEA: dehydroepiandrosterone

DHT: dihydrotestosterone

ECOG PS: Eastern Cooperative Group Performance Status

EMA: European Medicines Agency

FDA: Food and Drug Administration

FSH: follicle-stimulating hormone

HLG: hormona liberadora de gonadotrofina

HR: hazard ratio

IC: intérvalo de confianza

ICD: International Classification of Diseases

LH: luteinizing hormone

LHRH: luteinizing-hormone releasing hormone

MAB: maximum androgen blockade

mTOR: mammalian target of rapamycin

NR: not reached

OR: odds ratio

ORR: objective response rate

OS: overall survival

PCWG2: Prostate Cancer Working Group criteria 2

PFS: progression-free survival

PIN: prostatic intraepithelial neoplasia

PI3K: phosphatidylinositide 3-kinase

PR: partial response

PSA: prostate-specific antigen

RA: receptor de andrógenos

RECIST 1.1: Response Evaluation Criteria in Solid Tumours 1.1

SD: stable disease

SEER: National Cancer Institute Surveillance Epidemiology and End Results

SG: supervivencia global

SLP: supervivencia libre de progresión

SRAA: síndrome de retirada del antiandrógeno

SRE: skeletal-related event

TDA: deprivación androgénica

TCR: Thames Cancer Registry

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