GnRH agonists and the rapidly increasing use of combined androgen blockade in prostate cancer

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Abstract

The discovery of medical castration with GnRH agonists in 1979 rapidly replaced surgical castration and high doses of estrogens for the treatment of prostate cancer. Soon afterwards, it was discovered that androgens were made locally in the prostate from the inactive precursor DHEA of adrenal origin, a mechanism called intracrinology. Taking into account these novel facts, combined androgen blockade (CAB) using a pure antiandrogen combined with castration in order to block the two sources of androgens was first published in 1982. CAB was the first treatment shown in randomized and placebo-controlled trials to prolong life in prostate cancer, even at the metastatic stage. Most importantly, the results recently obtained with the novel pure antiandrogen enzalutamide as well as with abiraterone, an inhibitor of 17α -hydroxylase in castration-resistant prostate cancer, has revitalized the CAB concept. The effects of CAB observed on survival of heavily pretreated patients further demonstrates the importance of the androgens made locally in the prostate and are a strong motivation to apply CAB to efficiently block all sources of androgens earlier at start of treatment and, even better, before metastasis occurs. The future of research in this field thus seems to be centered on the development of more potent blockers of androgens formation and action in order to obtain better results at the metastatic stage and, for the localized stage, reduce the duration of treatment required to achieve complete apoptosis and control of prostate cancer proliferation before it reaches the metastatic or noncurable stage.

Key Words

- ▶ GnRH agonists
- ▶ intracrinology
- ▶ combined androgen blockade
- ▶ castration-resistant prostate cancer
- ▶ antiandrogens
- ▶ inhibitors of steroidogenesis

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Introduction

Prostate cancer is the most frequently diagnosed cancer and the second cause of cancer death in men in North America (Siegel *et al.* 2013). In fact, one in eight men will be diagnosed with prostate cancer during his lifetime. At such a rate, more than 3 000 000 men will die from prostate cancer among the male population presently living in the

United States, whereas more than 250 000 die annually worldwide from prostate cancer (Jemal *et al.* 2011). The very serious medical and social consequences of this disease are comparable to those of breast cancer in women.

Although improvements in surgery and radiotherapy have occurred, a study reported by Lichtenberg (2002)

using National Cancer Institute data from 2.1 million patients with cancer in the USA from 1975 to 1995 concluded that 'cancer-fighting drugs improved survival rates, especially for cancer of the prostate, where drug innovations have been the greatest'. The drug innovations in the field of prostate cancer have been gonadotropin-releasing hormone (GnRH) agonists (Labrie *et al.* 1980, 1996, 2005a) used to achieve medical castration and the pure antiandrogens or compounds having exclusive antagonistic activity at the androgen receptor (AR) (Neri *et al.* 1967, El Etreby *et al.* 1987, Furr *et al.* 1987) administered in association with medical or surgical castration (Labrie *et al.* 1982, 1985, Crawford *et al.* 1989, Labrie 2007). The combination of castration with a pure antiandrogen has been called combined androgen blockade (CAB).

Androgen blockade is the standard of care for advanced prostate cancer (Crawford 2009, Labrie 2011a), while the benefits of chemotherapy or immunotherapy are accompanied with more important side effects and are usually used at a later stage of the disease (Tannock *et al.* 2004, Eisenberger & Sinibaldi 2006, Kantoff *et al.* 2010).

It should be considered that despite the significant improvements in diagnosis and treatment, prostate cancer remains the second most common cause of death after lung cancer in American men. There is thus the need for potentially more potent, specific and well tolerated agents which can provide a longer and good quality of life while avoiding the morbidity associated with the progression of prostate cancer (Garcia & Rini 2012).

After briefly mentioning the current status of knowledge on the endocrinology/intracrinology of the prostate, this review will briefly summarize the data obtained after monotherapy (castration alone) and CAB given as first-line therapy at different stages of the disease. With regard to castration-resistant prostate cancer (CRPC), which is currently the main target of recent prostate cancer research, this review will refer to the proposed mechanisms of resistance to endocrine therapy and highlight the clinical observations which demonstrate that prostate cancer continues to respond to other forms of androgen blockade after development of resistance to blockade of testicular androgens, thus illustrating the major importance of extratesticular androgens made locally by the mechanisms of intracrinology.

The revival of the interest for CAB stems largely from the very encouraging clinical data obtained with a novel blocker of the AR (MDV-3100) and with an inhibitor of androgen formation (abiraterone) in CRPC patients. Extrapolation will then be made on potential future

developments in the field of androgen blockade and prostate cancer therapy. It should be remembered that prostate cancer is the most sensitive of all cancers to hormone therapy. Consequently, it is logical that every effort should be made to take advantage of this unique characteristic (Labrie 2011b).

Discovery of medical castration with GnRH agonists and its impact

More than 3 decades ago, when experimental animals were treated for a few days with a GnRH super-agonist, an increase in the weight of the seminal vesicles and prostate was expected. Unexpectedly, the opposite effect was observed: the prostate, seminal vesicles and testicles all became smaller after a few days of treatment (Auclair *et al.* 1977a, b, Pelletier *et al.* 1978).

Although experiments in rats did suggest that GnRH agonists had partial inhibitory effect on testicular function, we discovered that chronic administration of GnRH agonists achieves medical castration in men who appear to be the most sensitive of all species to the inhibitory effects of GnRH agonists on testicular androgen secretion. In fact, when we first administered a GnRH agonist to a patient with stage B prostate cancer, a 70–85% reduction in the serum levels of testosterone and dihydrotestosterone (DHT), respectively, occurred as early as 2 weeks after the start of therapy (Labrie *et al.* 1980; Fig. 1). The GnRH agonist used was buserelin at a dose of 500 µg given intranasally. Shortly afterwards, when the effects of various doses of buserelin administered intranasally and subcutaneously were compared in detail, results showed that the s.c. route should be preferred (Faure *et al.* 1982). Thirty-three years ago the first prostate cancer patient was treated with a GnRH agonist at the Laval University Medical Center in Quebec City, Canada, thus rapidly leading to the worldwide replacement of surgical castration and high doses of estrogens with GnRH agonists. The discovery of medical castration with GnRH agonists rapidly led to fundamental changes in the endocrine therapy of prostate cancer.

Medical castration with GnRH agonists was a somewhat a unique discovery in medicine since, for the first time, a completely unexpected inhibitory effect on testicular function was observed using a stimulatory molecule (GnRH agonist). The mechanism responsible for the castration effect of GnRH agonists is the loss of biological activity of luteinizing hormone (LH) during long-term treatment with GnRH agonists (St-Arnaud *et al.* 1986). According to the then current knowledge, a potent

Figure 1

Landmarks in the development of androgen blockade in prostate cancer.

GnRH agonist should have resulted in long-term and potent stimulation of testicular functions. This innovative therapy is particularly important for patients with localized or locally advanced prostate cancer who need a treatment for long-term use. Accordingly, GnRH agonists were rapidly adopted worldwide to achieve medical castration (Labrie *et al.* 2005a, Labrie 2007). The major medical importance of this discovery is indicated by worldwide annual sales of the order of US\$ 3.0 billion during the last 25 years. GnRH antagonists have been developed to achieve comparable inhibitory effects on testosterone secretion by the testicles.

Effect of castration on localized or locally advanced disease

Most importantly, the excellent tolerance observed with GnRH agonists has permitted a series of studies in localized/locally advanced disease which have shown a major reduction in the death rate due to prostate cancer, ranging, in the early studies, from 31 to 87% at 5 years of follow-up (see Labrie *et al.* (2005a) for review) (Labrie *et al.* 2005a). A meta-analysis that combined data from several studies involving ~5000 men concluded that the risk of dying from prostate cancer within 10 years decreased by one third if blockade of testicular testosterone secretion was started immediately after diagnosis of localized or locally advanced disease rather than once the disease had

progressed (Arnst 2003, Peto & Dalesio 2003). These data led (Peto & Dalesio 2003) to the conclusion that 'hormone treatment as a whole works much better than previously thought'. It should be remembered, however, that these results were obtained by blockade limited to testicular androgens. As indicated later, only 60–75% of total androgens present in the prostate were eliminated (Labrie *et al.* 2009). Knowing, as discussed later, the multiple sources of prostatic androgens, it is not surprising to believe that optimal treatment requires a combination of therapeutic agents or CAB at minimum.

Two sources of androgens are active in the human prostate: intracrinology

Humans and other primates are unique in having adrenals that secrete large amounts of the inactive precursor steroids DHEA and DHEA-S, which are converted into potent androgens in many peripheral tissues, including the prostate (Fig. 2). In adult men, the plasma concentration of DHEA-S secreted by the adrenals is 100–500 times as high as that of testosterone, the main secretory product of the testes (Labrie *et al.* 1985, 2005a). Therefore, there are large circulating amounts of the precursors required for the production of active androgens in the prostate and other peripheral intracrine tissues (Fig. 3).

A particularly remarkable and highly sophisticated achievement of evolution is intracrinology, the

Figure 2

Sources of androgens in the human prostate. Hypothalamic GnRH stimulates the release of LH from the anterior pituitary. LH, in turn, stimulates the Leydig cells of the testes to produce testosterone, which is released into the bloodstream. Pituitary ACTH stimulates glucocorticoid and DHEA secretion from the adrenal glands. DHEA is converted into

testosterone and then into DHT and some estrogens in the prostate. Each of the two pathways (testis and adrenal) provides comparable amounts of androgens. ACTH, adrenocorticotropin; DHT, dihydrotestosterone; GnRH, gonadotropin-releasing hormone; LH, luteinizing hormone.

mechanism that permits specific and local production of sex steroids for a strictly local action without significant release of active sex steroids in the circulation. In 1988 this mechanism was named as intracrinology (Labrie *et al.* 1988a) following observations made in the early 1980s in men castrated for prostate cancer (Labrie *et al.* 1985) which showed that an important proportion of the androgens present in the human prostate is made from the DHEA present in the prostate itself.

Through an estimated period of 500 million years (Baker 2004), evolution has progressively provided the peripheral tissues with the enzymes able to make and inactivate sex steroids locally (Fig. 3). However, it is only about 50 million years ago that the ability to secrete large amounts of the precursor substrate, DHEA, appeared in the adrenals of primates. Very recently, the transformation of DHEA into estrogens and/or androgens by the mechanisms of intracrinology has been demonstrated (Labrie *et al.* 1985, 1988a, 1989, 2005b, Labrie 1991, 2010a, Luu-The 2011; Figs 3 and 4).

It is very important to mention that an essential aspect of intracrinology is that the active sex steroids are

not only made locally but also inactivated locally at the same site where synthesis takes place (Bélanger *et al.* 2003; Fig. 4). In fact, the sex steroids made from DHEA in peripheral tissues are essentially released outside the cells as inactive compounds. (Labrie *et al.* 2009, Olsson *et al.* 2010).

As illustrated in Fig. 4, DHEA of adrenal origin is distributed by the general circulation to all tissues indiscriminately. The transformation of DHEA into estrogens/androgens, however, is tissue-specific, ranging from none in the endometrium to various levels in the other tissues of the human body. Most importantly, ~95% of the active estrogens and androgens are inactivated locally before being released into the blood as inactive metabolites, thus avoiding inappropriate exposure of the other tissues (Labrie 2010a; Fig. 4). The local intracellular inactivation of testosterone and DHT explains why only a very small fraction of the active androgens (and estrogens) secreted intracellularly are manifested in the blood stream (Figs 4 and 5A). In fact, serum levels of testosterone are reduced by 97% following castration in men aged between 69 and 80 years (Fig. 5; Labrie *et al.* 2009).

Figure 3

Human steroidogenic and steroid-inactivating enzymes in peripheral intracrine tissues: 4-dione, androstenedione; A-dione, 5- α -androstane-3, 17-dione; ADT, androsterone; epi-ADT, epiandrosterone; E₁, estrone; E₁-S, estrone sulfate; E₂, 17 β -estradiol; E₂-S, estradiol sulfate; 5-diol,

androst-5-ene-3 α , 17 β -diol; HSD, hydroxysteroid dehydrogenase; testo, testosterone; RoDH-1, Ro dehydrogenase 1; AR, androgen receptor; ER, estrogen receptor; UGT2B28, uridine glucuronosyl transferase 2B28; Sult2B1, sulfotransferase 2B1; UGT1A1, uridine glucuronosyl transferase 1A1.

The contribution of adrenal DHEA to total androgen levels is best illustrated by the concentration of the serum androgen metabolites remaining in the blood stream and by the concentration of intraprostatic DHT (the most potent naturally occurring androgen) remaining in the prostate, after castration (Labrie *et al.* 1985, 2009, Bélanger *et al.* 1989, Labrie 2008, 2010b; Fig. 5). The sum of the concentrations of androgen metabolites (the total androgen pool) is the only accurate method for measuring total androgenic activity in the circulation (Labrie *et al.* 2006). This sum of androgen metabolites is only reduced by about 60% after castration (Fig. 5). Such findings indicate that about 40% of total androgens in the prostate are made locally and thus remain in the prostate and free to stimulate prostate cancer after elimination of testicular androgens. These data are in close agreement with the measurements of intraprostatic DHT concentrations by different laboratories. These data show that, globally, the concentration of DHT measured in the prostate after castration approximately ranges from 20 to 50% of that measured in intact men (Fig. 5; Labrie *et al.* 1985, Bélanger *et al.* 1989, Nishiyama *et al.* 2004, Mostaghel *et al.* 2007).

Another study has shown that intraprostatic DHT levels remained at 50% of intact values after castration (Yoon *et al.* 2008).

Combined androgen blockade

A clear distinction must be made between monotherapy and CAB

Once the presence of two sources of androgens stimulating prostate cancer is known, it is important to use a precise terminology to be able to discriminate between the different sources of androgens which need different interventions to be controlled. In this context, androgen deprivation therapy (ADT) is an imprecise terminology which does not permit the identification of different treatment modalities having different efficacies. For example, 'surgical or chemical castration' should not simply be called 'ADT' because it does not discriminate between very different sources and blockages of androgens. The exact situation is rather the following: 'surgical or chemical castration has been the treatment of choice

X, very low subthreshold or biologically inactive levels in blood; do not exert significant systemic biological activity
XX, 95% or more eliminated as inactive metabolites

- (1) Labrie 1991 *Molecular and Cellular Endocrinology* **78**, C113–C116.
Labrie, Luu-The *et al.* 2005 *Journal of Endocrinology* **187**, 169–196.
Labrie, Martel *et al.* 2011 *Menopause* **18**, 30–43.

Figure 4

Schematic representation of the mechanisms of intracrinology: DHEA of adrenal origin is transported by the circulation to all peripheral tissues where it is transformed into cell-specific concentrations and ratios of testosterone and/or estradiol, depending upon the cell-specific pattern and level of expression of the steroid-forming and steroid-inactivating enzymes. Testosterone and estradiol are 95% or more inactivated locally, with a very small amount of active sex steroids diffusing into the blood where they are present at subthreshold or biologically inactive

concentrations, thus avoiding systemic effects in the other tissues, a condition necessary in postmenopausal women to avoid stimulation of the endometrium (Labrie & Labrie 2013). The inactive metabolites of testosterone and estradiol, especially glucuronides and sulfates, are released into the blood from which they are removed by the kidneys and liver. In fact, comparable serum sex steroid levels are observed in castrated men and postmenopausal women (Labrie *et al.* 2011).

for patients with advanced disease for the past 70 years'. As will be discussed later, ADT is not the synonym of surgical or chemical castration; the term ADT can also cover, among other possibilities, CAB, which is very different from castration alone.

Distinction must be made between localized and metastatic disease

The stage of the disease is an extremely important but an often neglected variable. A generalized and scientifically incorrect situation is to use conclusions obtained in metastatic prostate cancer and apply them to localized disease. The following is an example: 'prostate cancer is a disease that initially responds but later becomes resistant to androgen blockade' (Scher *et al.* 2012). As discussed in more detail later, this statement essentially comes from metastatic disease but is not appropriate for localized disease of which cure can be obtained with CAB (Labrie *et al.* 2002, Akaza 2006, Akaza *et al.* 2006a). Similarly, it

cannot be said 'All patients ultimately progress to metastatic CRPC' (Fizazi *et al.* 2012). This sentence is correct for patients first treated by castration at the metastatic stage, but it does not apply for CAB treatment at the localized stage of which cure is a possibility and progression is an exception (Labrie *et al.* 2002, Akaza *et al.* 2004, 2006a, Egawa *et al.* 2004, Homma *et al.* 2004, Akaza 2006, Ueno *et al.* 2006).

Treatment of metastatic disease

It is important to mention that no study has shown that medical or surgical castration alone had a statistically significant effect on survival in patients with metastatic prostate cancer. The data obtained for the first patients treated with CAB were published in 1982 (Labrie *et al.* 1982), thus leading to a series of randomized placebo-controlled studies, the first one being reported by Crawford *et al.* (1989), who showed statistically significant benefits on survival (Crawford *et al.* 1989). In fact, the

Labrie *et al.* 2009 *Journal of Steroid Biochemistry and Molecular Biology* 113, 52–56.

Figure 5

Effect of castration on serum androgen levels. (A) Castration reduces serum testosterone levels by 97%. (B) The total androgen pool (the sum of the androgen metabolites androsterone glucuronide, androstane-3 α -diol-3G and androstane-3 α -diol-17G) remains high at ~40% of the levels observed

in intact men. (C) Intraprostatic levels of dihydrotestosterone, the predominant androgen in the prostate, also remains at about 40% of the levels observed in intact men, an observation which indicates that the ~40% remaining DHT after castration has an extratesticular origin.

combination of a pure antiandrogen (flutamide) with a GnRH agonist was the first treatment shown to prolong life in patients with advanced prostate cancer (Labrie *et al.* 1982, Crawford *et al.* 1989).

All clinical trials in patients with advanced prostate cancer have shown that CAB has some significant advantages over castration alone. These advantages include a higher proportion of patients with complete and partial responses, improved control of pain associated with metastatic disease, longer disease-free survival, and longer overall survival (Crawford *et al.* 1989, Caubet *et al.* 1997, Denis *et al.* 1998, Bennett *et al.* 1999, Prostate Cancer Trialists' Collaborative Group 2000). A large-scale study comparing castration+flutamide as the initial treatment compared with castration alone at start of treatment but with flutamide added later (when the cancer progressed despite castration) (Eisenberger *et al.* 1998) did not show a statistically significant effect of CAB ($P=0.14$). This study was practically unanimously interpreted as showing no benefit while the data indicate an 86% likelihood that CAB administered at the start of treatment is superior on overall survival to administering flutamide later at time of relapse. It should also be mentioned that the above-mentioned study, like all the other clinical trials, did not compare CAB with castration alone but CAB with the addition of flutamide (or other androgen blocker) at time of progression after castration, an approach which has been found early to induce a positive response in 34.2% of patients progressing after castration (Labrie *et al.* 1988b).

CAB as a first-line treatment in metastatic disease increases overall survival by an average of 3–6 months compared with combined treatment with the antiandrogen started later (Crawford *et al.* 1989, Caubet *et al.* 1997, Denis *et al.* 1998, Bennett *et al.* 1999, Prostate Cancer Trialists' Collaborative Group 2000). Assuming that about half of these patients die from causes other than prostate cancer, this prolongation of life corresponds to an actual increase in life duration of 6–12 months when only prostate cancer is considered as cause of death.

While CAB, as mentioned above, has many advantages on quality of life (including rapid response, decrease in pain, and survival) (Crawford *et al.* 1989, Caubet *et al.* 1997, Denis *et al.* 1998, Bennett *et al.* 1999, Prostate Cancer Trialists' Collaborative Group 2000), the costs are higher than other treatment options and some data suggest the possibility of negative cardiovascular effects (Levine *et al.* 2010) which need to be further investigated and balanced with the benefits mentioned earlier (Labrie *et al.* 2002, Akaza *et al.* 2004, 2006a, b, Egawa *et al.* 2004, Homma *et al.* 2004, Akaza 2006, 2008, Ueno *et al.* 2006, Namiki *et al.* 2008).

Localized/locally advanced disease

Considering the increasing aggressiveness and genetic abnormality of cancer in progression, it is logical to believe that improved or much improved results should be obtained if CAB is started when the cancer is limited to

the prostate (localized) or is limited to the pelvic area (locally advanced) before the distant metastatic or irreversible stage occurs. Early data have indicated that the benefits of CAB are greater for patients with minimal metastatic disease than for those with extensive metastatic disease (Crawford *et al.* 1989, Denis *et al.* 1998). Similarly, in a study of 205 patients with stage C/D disease, at 5.2 years of follow-up, there was an advantage of CAB (GnRH agonist + 80 mg bicalutamide) over a GnRH agonist alone for overall survival ($P=0.04$) (Akaza *et al.* 2009). The effect on cancer-specific death had not reached statistical significance ($P=0.09$). These data support those of other studies which investigated CAB for treatment of stage C prostate cancer (Dupont *et al.* 1988, 1993a). In Japan, for 8424 cases of organ-confined or regional disease, initial treatment with CAB or castration alone was used in 39.9% of patients, radical prostatectomy in 38.1%, radiation therapy in 18.2%, and watchful waiting in 4.7% of cases (Fujimoto *et al.* 2011). Among the patients with nonmetastatic disease, 65% received CAB while 25% received monotherapy with a GnRH agonist or surgical castration.

A survival advantage of prostate cancer patients treated with radiotherapy with a GnRH agonist for 3 years compared with radiotherapy alone has been observed (Bolla *et al.* 2009). More recently, a GnRH agonist treatment given before (neoadjuvant) and during radiotherapy has shown decreased prostate cancer mortality and increased overall survival (Jones *et al.* 2011). These data indicate that a better response to androgen blockade is observed if treatment, albeit being partial and suboptimal, is applied at a locally advanced stage. As mentioned earlier, no survival benefit has ever been observed with castration alone at the metastatic stage.

It is important to mention that if treatment is initiated immediately after diagnosis in patients with localized or locally advanced disease and is continued for at least 7 years without interruption, CAB can achieve long-term control or potentially cure prostate cancer in at least 90% of cases, as judged by no increase in serum prostate-specific antigen (PSA) within at least 5 years after stopping CAB administered up to 9 years previously (Labrie *et al.* 2002).

It is often erroneously mentioned that androgen blockade should not be administered early in prostate cancer because resistance to treatment is very likely to develop, thus 'saving' androgen blockade for use at a later stage of the disease. The fact that this belief is incorrect is proven by the observation that resistance to CAB develops extremely rarely in patients with localized prostate cancer (Labrie *et al.* 2002, Akaza *et al.* 2006a, Ueno *et al.* 2006), while resistance to CAB develops in practically all patients

having metastatic disease at the start of treatment. In fact, as mentioned above, resistance to CAB is a problem specific to metastatic disease and is a rare observation when CAB is started at the localized stage.

Castration-resistant prostate cancer

The unavoidable reactivation or progression of prostate cancer in patients with metastatic disease originally treated by castration alone has, until recently, been considered androgen-independent or hormone-refractory prostate cancer. It has become clear, however, as suggested by the benefits observed after addition of flutamide to patients with prostate cancer progressing after castration (Labrie *et al.* 1988b) that the cancer progressing after castration remains androgen driven. More recently, these observations have been strongly revitalized, thus leading to the exciting development of MDV-3100 (Tran *et al.* 2009) and abiraterone (Attard *et al.* 2008) in CRPC patients.

Mechanisms of resistance to castration

The best scientifically supported mechanisms proposed to explain the response to further androgen blockade in CRPC patients are the local intraprostatic production of androgens, increased sensitivity to low androgens, AR amplification, splice variants, and mutations of AR leading to spontaneous AR activity or promiscuity with abnormal ligands (Labrie 2011a, Massard & Fizazi 2011, Mostaghel & Plymate 2011).

The human prostate contains the enzymes that convert DHEA into active androgens (Labrie *et al.* 1988a, El-Alfy *et al.* 1999, Nakamura *et al.* 2005, Luu-The *et al.* 2008, Pelletier 2008, Evaul *et al.* 2010). Although the serum levels of DHEA-S in the blood are much higher than those of DHEA, the intraprostatic concentrations of DHEA and DHEA-S are similar (Mohler *et al.* 2004). As mentioned above and as shown in Fig. 5C, intraprostatic DHT remains at sufficiently high levels to activate the AR following castration (Labrie *et al.* 1985, Bélanger *et al.* 1989, Mizokami *et al.* 2004, Nishiyama *et al.* 2004). Somewhat lower levels of intraprostatic DHT have been more recently measured after castration, but the authors always indicate the importance of the remaining DHT for continuous cancer progression after castration and the role of intracrine androgen formation (Titus *et al.* 2005, Page *et al.* 2006, Arai *et al.* 2011).

Prostate cancer progressing in castrated patients is dependent on the activation of AR by androgens (Chen *et al.* 2004, Mohler *et al.* 2004), as well demonstrated by the

response of CRPC by blockade of AR with the antiandrogen MDV-3100 which prevents DHT from binding to AR and its transfer to the nucleus where it can stimulate transcription of the androgen-responsive genes (Tran *et al.* 2009) and the inhibitor of 17 α -hydroxylase abiraterone (Attard *et al.* 2008). In most CRPC cases, the AR protein and AR-regulated genes are expressed, indicating that AR is still active and likely to be stimulating tumor growth (van der Kwast *et al.* 1991, Holzbeierlein *et al.* 2004, Scher & Sawyers 2005, Yuan & Balk 2009). In fact, AR is overexpressed in the majority of CRPC patients, thus potentially making prostate cancer cells hypersensitive to androgens (Linja *et al.* 2001). AR can also be mutated, thus causing AR to be activated by steroids other than testosterone and DHT (Taplin *et al.* 2003).

A suggested source of testosterone in CRPC is cholesterol (Twiddy *et al.* 2011). LNCaP cells have been reported to be able to transform radiolabeled cholesterol into testosterone *in vitro* and *in vivo* (Dillard *et al.* 2008, Locke *et al.* 2008, 2009). It has also been reported that CYP17A1 expression was increased in CRPC samples from patients treated with ketoconazole (Cai *et al.* 2011). It should be mentioned, however, that the mRNA levels of the steroidogenic enzymes required for complete *de novo* steroid synthesis from cholesterol are very low or absent in human prostate cancer cell lines and in the majority of clinical prostate cancer samples (Hofland *et al.* 2010) while the enzymes converting DHEA or 4-dione into androgens were elevated and even increased in CRPC patients (Stanbrough *et al.* 2006).

It is of interest that a correlation was found between the decline in serum PSA during androgen blockade and the initial intraprostatic androgen levels (Shibata *et al.* 2013). Both androgens, made locally from DHEA with a possible contribution of androgens made locally from cholesterol, can contribute to the intraprostatic androgens remaining after castration (Labrie *et al.* 1985, Mohler *et al.* 2004, Arnold *et al.* 2005, Stanbrough *et al.* 2006, Locke *et al.* 2008, Montgomery *et al.* 2008, Sharifi & Auchus 2012).

Most importantly, the intraprostatic concentration of DHEA has been measured by liquid chromatography tandem mass spectrometry at values of the order of 30 ng/g tissue, with no change after castration/hormone therapy (Arai *et al.* 2011). These values of intraprostatic DHEA are five- to tenfold higher than the DHEA concentration in the blood (Labrie *et al.* 2009) and similar to the concentration of DHEA measured in benign and cancerous breast tissue in women (Poortman *et al.* 1983). Such data suggest that the presence of

mechanisms permits DHEA transfer from the blood to the prostatic tissue, thus providing relatively high concentrations of intraprostatic DHEA available for transformation into testosterone and DHT by the intracrine mechanisms (Fig. 3).

The important clinical responses observed with the antiandrogen MDV-3100 and the 17 α -hydroxylase inhibitor abiraterone are clear demonstrations of the importance of androgens made and acting in the prostate according to the mechanisms of intracrinology. The enzymatic conversion of DHEA to DHT has been shown to be essential for the activation of AR in LNCaP prostate cancer cells (Evaul *et al.* 2010), while *in vitro* studies have shown that prostate cancer stromal cells and human LNCaP prostatic cancer cells coordinate activation of AR via synthesis of testosterone and DHT from DHEA (Mizokami *et al.* 2009). In addition, increased expression of genes that code for the enzymes that convert DHEA into testosterone has been observed in CRPC (Stanbrough *et al.* 2006), while cancerous prostatic tissue can synthesize more DHT than does benign prostatic tissue (Nishiyama *et al.* 2007).

Interestingly, in this context, testosterone levels have been reported to be higher in metastases of prostate cancer from anorchid men than they are in primary cancers obtained from untreated eugonadal men (Mizokami *et al.* 2004). This finding could be explained by dysregulated expression of the genes that encode steroidogenic enzymes in cancer cells, a well-known phenomenon due to aberrant control of gene expression in cancer tissue. In addition to the local and autonomous synthesis of androgens, increased AR levels could explain the observation that androgen deprivation in prostate cancer xenograft models results in only transient cell cycle arrest (Agus *et al.* 1999). In fact, xenograft tumors show little evidence of apoptosis, and frequently grow rapidly, despite androgen deprivation. In preclinical models, overexpression of AR shortens the response duration and makes the tumors resistant to bicalutamide (Chen *et al.* 2004).

Sensitive liquid chromatography–tandem mass spectrometry has identified 5 α -dihydrodeoxy-corticosterone (5 α -OH-DOC) in prostate cancer tissue of CRPC patients, a compound which could activate AR and stimulate cancer cell growth (Uemura *et al.* 2010). On the other hand, by reducing intraprostatic androgens, abiraterone has been reported to induce the appearance of AR splice variants (Mostaghel *et al.* 2011) with the possibility of ligand-independent activation of AR (Mostaghel & Plymate 2011).

It is worth noting that low serum testosterone (Karamanolakis *et al.* 2006, Morgentaler & Rhoden 2006) and intraprostatic DHT (Nishiyama *et al.* 2006) have been associated with increased risk of prostate cancer and higher Gleason score. Other studies have shown low serum testosterone to be associated with more aggressive disease (Ribeiro *et al.* 1997), more advanced stage at radical prostatectomy (Massengill *et al.* 2003, Isom-Batz *et al.* 2005), and higher grade cancer (Schatzl *et al.* 2001). In this context, it is possible that the partial 50–80% reduction in intraprostatic DHT observed following castration alone could trigger the same negative mechanisms, thus providing an explanation for the well recognized difficulty in treating CRPC. Such possibility can be supported by the findings of increased androgen biosynthesis accompanying low AR levels (McNamara *et al.* 2013).

It is of interest to mention that in the study of Nishiyama *et al.* (2006), the low intraprostatic DHT was

not correlated with serum testosterone but with serum DHEA (Nishiyama *et al.* 2006), thus suggesting that intraprostatic DHT was modulated significantly by local intraprostatic formation of DHT from circulating DHEA by the intracrine mechanisms. Further support for the importance of extratesticular androgens is provided by the finding of a prostatic carcinoma in a patient 22 years after orchiectomy (Sharkey & Fisher 1960).

Animal studies (Rosner *et al.* 1969) and human fetal studies (Voutilainen & Miller 1986) indicate that DHEA and androgens are produced in nongonadal tissues other than the adrenal glands. Consequently, in analogy with other peripheral tissues, it is possible that DHEA could be synthesized from cholesterol to an unknown extent in the prostate (Mostaghel *et al.* 2012; Fig. 6). The potential local source of androgens possibly derived from cholesterol could be implicated, up to an unknown extent, in the development and progression of CRPC by acting directly on prostatic AR.

Figure 6

Potential sites of action for hormonal therapies in prostate cancer. On average, 60–75% of intraprostatic DHT is made from testosterone of testicular origin, highlighting the major importance of castration in the treatment of prostate cancer (1). The remaining intraprostatic DHT is made locally from the adrenal precursor DHEA (2). Some DHT could possibly be made locally from cholesterol (3). Accordingly, blocking the action of DHT made locally in the prostate after castration with a pure antiandrogen has an importance practically comparable with castration itself (4). In patients

with CRPC, increased AR levels (5), mutated AR (5), and increased intraprostatic DHT formation (2, 3) seem to be implicated in disease progression. Potential changes in the normal equilibrium of the AR-ligand-co-activators-co-repressors complex (6, 7) could also be involved. Mutated AR could render some endogenous steroids stimulatory to AR action (8). AR, androgen receptor; CRPC, castration-resistant prostate cancer; DHT, dihydrotestosterone.

Table 1 Castration-resistant prostate cancer treated with chemotherapy

	Overall survival (months)	Hazard ratio for death	Progression-free survival (months)	Hazard ratio for progression
Enzalutamide ^a vs placebo	18.4 vs 13.6	0.63	8.3 vs 2.9	0.40
Abiraterone ^b + prednisone vs prednisone alone	15.8 vs 11.2	0.74	5.6 vs 3.6	0.66

^aScher *et al.* (2012).^bFizazi *et al.* (2012).

CAB in CRPC

For patients with CRPC, the choice of treatments has been limited, although many responses were known to be obtained with further androgen blockade after castration (Labrie *et al.* 1985, 1988b, Small & Vogelzang 1997).

As mentioned above, when androgen blockade is started at the metastatic stage, disease progression always occurs. Such resistance to treatment has generally been viewed as the end of the therapeutic role of androgen blockade. However, as mentioned earlier, early preclinical (Labrie & Veilleux 1986) and clinical (Labrie *et al.* 1985, 1988b) data clearly indicated that responsiveness to androgens remains present at all stages of prostate cancer, thus providing an opportunity to develop effective hormonal therapies for these patients. In a series of nine studies carried out in patients progressing after castration (CRPC), a decrease in 50% of PSA or more was seen in 6–66% of patients (Suzuki *et al.* 2010).

It is also of interest to mention that a large proportion of patients experience a paradoxical positive clinical response upon a simple arrest of antiandrogen administration or change in antiandrogen, with a decrease of >90% in serum PSA in up to 47% of patients (Collinson *et al.* 1993, Dupont *et al.* 1993b, Kelly & Scher 1993). Such data suggest that the ligand-specific changes in the 3D conformation of AR induced by each antiandrogen lead to preferential binding of different co-activators and co-repressors to AR, thus changing the activity of AR on transcription (Fig. 6).

Abiraterone

Abiraterone, by inhibiting the enzyme CYP17, decreases DHEA formation. It does also inhibit cortisol synthesis, thus stimulating ACTH secretion and adrenal mineralocorticoid secretion. The hypertension and hypokalemia

secondary to excess mineralocorticoid secretion can be attenuated by the co-administration of glucocorticoids (ex: prednisone) which decrease ACTH secretion (de Bono *et al.* 2011).

In a multicenter phase III clinical trial in which CRPC patients showed progression after docetaxel chemotherapy, overall survival after a median follow-up of 20.2 months was 15.8 months in the abiraterone+prednisone-treated group vs 11.2 months in the prednisone only group (Fizazi *et al.* 2012; Table 1). Mean radiologic progression-free survival was 5.6 vs 3.6 months for a hazard ratio of 0.66 in favor of abiraterone. For median time to PSA progression, values of 8.5 and 6.6 months were observed, for a hazard ratio of 0.63 in favor of abiraterone.

In CRPC patients not previously treated with chemotherapy, abiraterone+prednisone increased radiographic progression-free survival to 16.5 months compared with 8.3 months for the men receiving prednisone alone for a 47% reduction in the progression hazard after a median follow-up of 27.1 months (Ryan *et al.* 2013; Table 2). Median overall survival was 35.3 months in the abiraterone group compared with 30.1 months in the control group, thus representing a 21% improvement with a hazard ratio of 0.79 in favor of abiraterone (Table 2; Rathkopf & Scher 2013). It can be mentioned that 69% of patients who received abiraterone+prednisone had at least a 50% decline in serum PSA compared with 29% with prednisone alone. Significant advantages were also observed of time to chemotherapy, opiate use, PSA progression, pain progression, and deterioration in health-related quality of life. The benefits observed with abiraterone in addition to the known effects of prednisone (Tannock *et al.* 2004, Sternberg *et al.* 2009) demonstrate the importance of extratesticular androgen biosynthesis and, consequently, the importance of CAB.

The most common adverse events accompanying abiraterone treatment are associated with increased mineralocorticoid secretion, namely hypokalemia,

Table 2 Castration-resistant prostate cancer not treated with chemotherapy

	Overall survival (months)	Hazard ratio for death	Progression-free survival (months)	Hazard ratio for progression
Abiraterone+ prednisone	35.1	0.79	16.5	0.53
Prednisone	30.1	0.79	8.3	0.53

Ryan *et al.* (2013). Rathkopf & Scher (2013).

hypertension, and fluid retention, which are mitigated by concomitant prednisone administration (de Bono *et al.* 2011, Fizazi *et al.* 2012).

Enzalutamide

MDV-3100 (enzalutamide) is a novel antiandrogen with no agonistic activity (Tran *et al.* 2009, Jung *et al.* 2010). In 1199 CRPC patients having received chemotherapy with docetaxel, the median overall survival was 18.4 months in the group of men treated with enzalutamide (160 mg/day) compared with 13.6 months in men started on placebo, thus leading to a 37% decrease in the risk of death from any cause (Table 1). The hazard ratio for death after 520 deaths had occurred was 0.63. The superiority of enzalutamide was also shown for all secondary endpoints: radiographic progression-free survival was reduced from 8.3 months in the placebo group to 2.9 months in patients treated with enzalutamide for a hazard ratio of 0.40 (Table 1). Similar effects were observed for time to PSA progression: 8.3 vs 3.0 months with a hazard ratio of 0.25. (Scher *et al.* 2012).

Convulsions are a dose-dependent toxic effect of enzalutamide at doses above the therapeutic range observed in animals (Foster *et al.* 2011). Seizures were reported in phase 1–2 clinical trials with enzalutamide beginning at doses of 360 mg enzalutamide or more. Seizures were reported in 0.6% of patients in the phase III trial (Scher *et al.* 2012).

Conclusions

While more complete androgen blockade has been reported for quite some time to provide benefits in CRPC (Labrie *et al.* 1985, 1988a), the recent studies using the antiandrogen MDV-3100 (Tran *et al.* 2009, Scher *et al.* 2012) and abiraterone (Attard *et al.* 2008, de Bono *et al.* 2010, Fizazi *et al.* 2012) are the first placebo-controlled studies which provide a precise measure of the amplitude of the benefits achieved on progression-free survival and overall survival in CRPC. The addition of 4–5 months of overall survival obtained with MDV-3100 (Scher *et al.* 2012) and abiraterone (Fizazi *et al.* 2012) in CRPC patients confirms the central role of androgens and AR through all stages of prostate cancer. In fact, even after chemotherapy, the tumor progressing after castration is still dependent upon androgens and respond significantly to MDV-3100 and abiraterone. These crucial data stress the importance of targeting AR signaling as a key therapeutic target at all stages of prostate cancer, even in patients

progressing following previous treatment with different androgen blockade manipulations.

Future avenues of research and development in androgen blockade

Future research on the physiological mechanisms of androgen biosynthesis, especially the local intracrine intraprostatic formation of androgens, is crucial in order to target with optimal precision each element involved in intraprostatic androgen formation (Fig. 6). The easy part of androgen blockade relates to the androgens of testicular origin which can be easily eliminated by medical (GnRH agonists or antagonists) or surgical castration. The much more difficult part relates to the control of the androgens made locally in the prostate by intracrine mechanisms.

Abiraterone has well demonstrated the importance of inhibiting androgen intraprostatic formation, while enzalutamide has shown the impact of blocking access to the AR following castration in progressing metastatic disease. The more potent and specific will be the inhibitors of androgen formation and action (anti-androgens), better should be the benefits of treatment. Priority should thus be devoted to the availability of well-tolerated and more potent inhibitors of steroidogenesis and more potent and exclusively antagonistic blockers of AR.

The data obtained with enzalutamide and abiraterone in patients with metastatic disease progressing after castration are playing a crucial role in our understanding of the intraprostatic formation of androgens (intracrinology) and provide the enthusiasm to move further. Accordingly, with today's knowledge, CAB should be given as first-line therapy in metastatic disease, or as early as possible before metastasis occurs. In fact, it seems clear that for prostate cancer, in analogy with any other type of cancer, the earlier the treatment, the higher should be its success with even the possibility of a cure in localized disease (Labrie *et al.* 2002, Akaza 2005).

Clinical research should therefore be directed to treatments applied at the localized stage of the disease when cure is a possibility (Labrie *et al.* 2002, Akaza 2005). In analogy with metastatic disease, it will then be important to use the most potent antiandrogens and/or inhibitors of androgen biosynthesis in order to reduce the duration of treatment required to obtain complete apoptosis or elimination of localized disease. It should be indicated that even with today's imperfect drugs, application of the knowledge gained with intracrinology (Labrie 1991) and the resulting development of CAB (Labrie *et al.* 1982, 1993, Crawford *et al.* 1989,

Attard *et al.* 2008, Tran *et al.* 2009), elimination of death from prostate cancer is a possibility. More potent drugs should make this objective easier to achieve at an earlier stage of the disease.

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