Continued targeting of androgen receptor signalling: a rational and efficacious therapeutic strategy in metastatic castration-resistant prostate cancer

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Background

Prostate cancer represents the most common cancer in men, leading to 11% of all male cancer-related deaths [1]. For the last few decades patients with metastatic prostate cancer with a rising prostate-specific antigen (PSA) while on treatment with luteinising hormone-releasing hormone (LHRH) analogues, have been classified as "hormone refractory", to define the development of resistance to surgical or medical castration and in general to hormonal treatments [2]. Following this, prostate cancer therapy has been limited to first-line taxotere-based chemotherapy or secondary hormonal manipulations. Two prospective randomised trials have shown that docetaxel prolongs life by 2–3 months compared with mitoxantrone and improves the quality of life and symptom control in patients who have progressed on androgen deprivation therapy (ADT) [3,4]. The alternative unsatisfactory options comprised diethylstilbestrol, anti-androgens, low-dose steroids and ketoconazole, all characterised by a short-lasting (4 months) response in approximately 20–30% of patients and no proven impact on overall survival (OS) [5].

Over the past decade, a strong body of laboratory and clinical evidence has completely changed our understanding and the therapeutic approach to advanced prostate cancer, which remains hormone-driven, despite progression in medical or surgical castration. It is for this reason that nowadays the correct term used to describe this condition is "castration-resistant prostate cancer" (CRPC) and is no longer called "hormone-refractory prostate cancer", as it was before [6,7]. This misconception was based on the clinical experience of a poor response to secondary hormonal manipulations following progression in ADT. To support this concept it was hypothesised that prostate cancer might be composed of a heterogeneous population of cancer cells, some of which are androgen-independent and therefore more likely to survive and dominate in the context of an androgen-deprived tumour environment [8]. However, more recent laboratory research has revealed that following the selective pressure of androgen deprivation, in the majority of prostate cancer cells there is overexpression of the androgen receptor (AR) [9] and increased expression of enzymes responsible for the intratumoural synthesis of androgens [10].

The centrality of AR-signalling in the proliferation and survival of prostate cancer cells is indirectly demonstrated by the increased expression of androgen-regulated genes such as PSA concomitantly with disease progression [11]. Furthermore, clinical data from novel AR-targeting agents have shown that the effective targeting of AR signalling following the castration-resistant status results in a biochemical and radiological response suggesting that reactivation of AR signalling is the biological event underlying progression, despite castration levels of testosterone.

Abiraterone acetate, a novel potent selective inhibitor of CYP17, a key enzyme along the biosynthesis of androgens and oestrogens, has demonstrated significant tumour activity (PSA decline $\geqslant$50% in 50–60% of both chemotherapy-naive and chemotherapy-refractory metastatic CRPC patients and radiological response) [12–16]. Notably, most of the patients enrolled were progressing after treatment with secondary hormonal manipulations. The same scenario was also shown in Phase II trials investigating the treatment activity of a novel anti-androgen, MDV3100, in metastatic CRPC patients [17].

These very promising preliminary data prompted further analysis in Phase III trials in pre- and post-docetaxel settings to test the effect on OS in patients treated with abiraterone and MDV3100. The results of the Phase III randomised placebo-controlled trial evaluating abiraterone acetate in comparison with placebo, both combined with prednisone, were presented at the ESMO (European Society of Medical Oncology)

Conference in Milan in October 2010. A significant improvement in OS of approximately four months was shown in metastatic CRPC patients treated with abiraterone acetate compared with placebo after failure of conventional chemotherapy with taxotere.

A randomised, double-blind, placebo controlled Phase III study for metastatic post-chemotherapy CRPC patients comparing MDV3100 versus placebo has also been completed and the results are awaited. A similar study investigating MDV3100 in the pre-chemotherapy setting is currently ongoing.

The successful data from these novel hormonal therapies have triggered the development of new similar, but potentially more sophisticated drugs targeting the AR signalling axis, which are currently in early clinical studies.

Androgens and androgen receptor signalling

The normal prostate and prostate cancers depend on a critical level of androgens to survive and proliferate. The main circulating androgen is testosterone, the majority of which (80–90%) is the terminal product of the hypothalamus–pituitary–gonadal axis and the remaining amount derives from the peripheral conversion of adrenal steroids [18]. The precursor of androgens is cholesterol, which is converted into different steroid hormones by multiple enzymes belonging to the cytochrome P450 (CYP) family in the adrenal glands and in the testes. The first reaction is catalysed by the enzyme CYP11A1, which transforms cholesterol into pregnenolone. This is converted into 17-hydroxypregnenolone first and then into dehydroepiandrosterone (DHEA) respectively by the 17-hydroxylase and the 17, 20-lyase activities of CYP17A1, a key enzyme with dual activity.

The final product of this enzymatic chain, testosterone, is converted and activated to dihydrotestosterone (DHT) by 5-α-reductase, an intracellular enzyme. DHT leads to its biological effects by interacting with the AR, a ligand activated transcription factor and member of the steroid hormone receptor (SHS) subfamily of nuclear receptors [19]. Structurally, AR is composed of three different domains: an aminoterminal-activating domain, a carboxy-terminal ligand binding domain and a central region containing two zinc fingers, which bind to the DNA. When the AR is not bound to its ligand, it is functionally inactivated and protected from degradation through the combination with heat-shock proteins (HSPs). The molecular interaction of AR with DHT induces a conformational modification that dissociates the HSPs and favours the phosphorylation of the AR, which then translocates into the nucleus and interacts with the androgen-responsive elements (AREs) located within the promoter regions of the target genes. In order to initiate the transcription of these target genes the activated AR requires the recruitment of co-activator proteins that combine with the AR complex [19].

The AR is continuously expressed and activated during the natural history of prostate cancer from the early stage of the organ-confined tumour to the metastatic stage and before and after progression to castration [20,21]. However, the biological effect of ADT, consisting of the inhibition of the gonadal androgen synthesis, is insufficient to completely deprive prostate cancer tumour cells of testosterone, which is still produced by the adrenal glands and by the tumour itself. Lowering circulating testosterone levels are initially effective at blocking tumour growth, but invariably become inadequate and prostate cancer progresses despite this.

Several mechanisms of resistance to hormonal therapies have been revealed, including the amplification and overexpression of AR, point mutations in correspondence with the ligand-binding region of AR with consequent activation by alternative ligands such as deoxycorticosterone, corticosterone and cortisol, ligand-independent constitutive activation of AR, constitutive activation of modified AR lacking the ligand-binding domain, *de novo* intratumoural synthesis of androgens secondary to upregulation of the enzymes involved in the steroidogenesis and finally the upregulation of different molecular pathways leading to survival and growth of prostate cancer cells, bypassing the AR signalling [9,22–26].

Epigenetic alterations, in particular acetylation, seem to play an important role in regulating the AR activity. The recruitment of co-activators and co-repressors can influence the transcriptional activity of AR by regulating the acetylation of androgen-responsive genes or the AR itself with their activity of histone deacetylase (HDAC) or histone acetyl transferase (HAT) [27].

Targeting the *de novo* intratumoural synthesis of androgens

On the basis of the observation that CYP17A1 and in particular the 17, 20-lyase activity appears to be highly upregulated in prostate cancer metastases [28], novel effective drugs have been developed or are currently in development to counteract the reactivation of AR signalling depending on intratumoural androgens.

Ketoconazole, an imidazole antifungal, is a reversible and non-specific inhibitor of the enzyme CYP17A1. However, the low affinity of this drug for CYP17A1 required high doses, which led to significant side effects, limiting the clinical utility of this compound [29].

More than one decade ago, a potent selective and irreversible inhibitor of CYP17A1, abiraterone acetate, was discovered at our institution by one scientist [30]. However, the potential risk of adrenal insufficiency refrained from further clinical development at that time. These concerns were contradicted by case reports of young patients affected by congenital CYP17 deficiency as they did not present adrenocortical insufficiency, showing only hypertension and pseudohermaphroditism [31]. In fact, the inhibition of CYP17 results in a compensatory increase in the adrenocorticotropic hormone (ACTH), which leads to an increased level by 10 to 40 times of glucocorticoid hormones, deoxycorticosterone and corticosterone. Subsequent interest from the pharmaceutical company Cougar & Cougar together, with the scientific belief that the drug was safe and active, allowed the initiation of the first clinical trials [7,12,13].

The phase I/II study investigated the safety and activity of continuous, daily, single-agent oral administration of abiraterone acetate in chemotherapy-naive patients with metastatic castration-resistant prostate cancer. Fifty to sixty percent of these patients had a decline in prostate-specific antigen (PSA) by $\geqslant$50% with a median time to PSA progression by PSAWG criteria (Prostate-Specific Antigen Working Group Criteria I) of 230 days. Similar activity was also shown in other Phase I/II trials in the post-chemotherapy setting, suggesting that prostate cancer remains hormone-driven following chemotherapy [14,16]. Radiological response was also observed in both groups of patients, 37% chemotherapy-naive and 27% chemotherapy-refractory. No dose-limiting toxicity was observed and the drug remained active at all doses tested from 250 mg to 2000 mg daily. However, 1000 mg was the recommended dose chosen for further analysis.

Approximately two-thirds of patients treated with abiraterone acetate developed three main side effects consisting of hypertension, hypokalemia, and fluid retention as a result of the secondary hypermineralcorticoid effect induced by the steroids upstream of CYP17. These side effects were well managed with the administration of a specific mineralcorticoid receptor antagonist eplerenone escalating the dose from 50 mg to 200 mg daily and the addition of dexamethasone to treat these toxicities was rarely required [32]. However, it has been shown that steroidal mineralcorticoid antagonists, such as eplerenone or spironolactone can act as agonists of the AR and it would be therefore preferable to avoid their use to prevent AR signalling reactivation [33].

Interestingly, one quarter of patients receiving dexamethasone (0.5 mg daily) at some point along with the treatment with abiraterone had a PSA response (defined as a 50% PSA decline since the start of steroids) and showed a prolonged duration of response to abiraterone. The biological mechanism underlying these responses is probably secondary to the reduced positive feedback of ACTH stimulating the adrenal glands to produce steroids that can potentially activate a mutated promiscuous AR in prostate cancer cells. The same mechanism explains the protective effect of low-dose dexamethasone (0.5 mg daily) or prednisolone (5 mg twice a day) against the hypermineralcorticoid effect (hypertension, hypokalemia and fluid retention) observed with abiraterone.

Taking these concepts into account, the design of the subsequent studies has combined abiraterone with prednisolone. However, considering the significant duration of response of abiraterone observed in early clinical trials, a possible pitfall of this regimen is represented by the need to manage, in the long term, the steroid-induced side effects. In this regards alternative mineralcorticoid receptor antagonists without agonist effect should be further assessed in order to minimise toxicity and allow long-term patient compliance.

Following these very encouraging results a Phase III randomised, double-blinded, placebo controlled trial was carried out recruiting 1197 patients with docetaxel-refractory metastatic CRPC from April 2008 till July 2009. The results of this trial were recently presented at the ESMO Conference in Milan showing that the combination of abiraterone plus prednisolone significantly increased median survival compared with placebo plus prednisolone (median survival time 14.8 versus 10.9 months; hazard ratio [HR], 0.65; $P < 0.0001$) [34]. A similar Phase III randomised, placebo controlled study investigating the efficacy of abiraterone combined with prednisolone compared with placebo plus prednisolone in chemotherapy-naive, asymptomatic or mildly symptomatic patients with metastatic CRPC, has completed the accrual and results will be soon available.

In an attempt to ameliorate the safety and tolerability of abiraterone acetate, maintaining or even increasing its efficacy, several new drugs have been introduced and are currently being investigated in early clinical trials.

TAK-700 is a novel irreversible, inhibitor of CYP17 with a certain degree of selectivity for the 17,

20-lyase activity of the enzyme. In theory, the selective inhibition of 17, 20-lyase would preserve the activity of the 17-hydroxylase resulting in the absence of the characteristic secondary hypermineralcorticoid excess observed with abiraterone. A phase I/II study has shown promising preliminary data and a Phase III trial investigating the efficacy of TAK-700 in chemotherapy pre-treated metastatic CRPC is currently ongoing [35].

To combine the effect of CYP17 inhibitors with androgen receptor antagonists a new drug, TOK-001 was selected through a drug screen and is being investigated in Phase I/II trials [36].

Androgen receptor antagonists

Asymptomatic patients progressing by PSA during treatment with ADT are generally treated with several hormonal manipulations before receiving the standard first line taxotere-based chemotherapy regimen.

Anti-androgens such as bicalutamide, flutamide and nilutamide have been used for more than 30 years as single agents or in combination with LHRH analogues to constitute the maximum androgen blockade (MAB). These agents combine with the AR at the level of the ligand-binding domain, preventing the binding of DHT and impairing the ability of the AR complex to recruit co-activators and therefore to effectively regulate the androgen-dependent targeted genes transcription [37,38]. However, these first-generation anti-androgens can only achieve a short-lasting modest response (less than 4 months), which seems to be largely affected by the relatively low affinity of these agents for the AR compared with DHT and by their partial agonistic effect [9].

In an attempt to overcome these limitations, new-generation anti-androgens have been introduced, showing higher affinities for AR, lack of AR agonist effect, and antitumoural activity in bicalutamide-resistant and AR-overexpressing prostate cancer cells [39]. MDV3100 is a novel, orally available pure anti-androgen, which blocks AR nuclear translocation, DNA binding and co-activator function. Promising results have been shown in Phase I/II studies with a PSA decline of >50% observed in 50 to 60% of pre- and post-chemotherapy patients [17]. The main side effect was the appearance of epileptic seizures at doses above 340 mg/daily, but no serious adverse events were observed at dose levels inferior to 240 mg/daily and therefore 160 mg/daily was chosen as the recommended dose for further clinical trials. A randomised, double-blinded, placebo-controlled trial investigating the efficacy of MDV3100 versus placebo in metastatic CRPC patients has recently closed the accrual and the results are awaited. A similar Phase III study is currently ongoing in the pre-chemotherapy setting.

Conclusion

The significant survival advantage demonstrated with abiraterone acetate in patients with metastatic CRPC in the post-chemotherapy setting has provided the clinical evidence that targeting AR signalling is a rational and efficacious approach. In response to the compelling evidence that prostate cancer remains hormone-driven even after failure of several hormonal manipulations and chemotherapy, there has been an explosion of new drugs targeting the AR signalling with the intent to overcome the multiple mechanism of resistance induced by the androgens-depleted tumour microenvironment. Sequential use of AR signalling targeting new agents with different mechanisms of action is likely to counteract the AR signalling reactivation, which is the biological basis of most cases of progression.

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Conflict of interest statement

J.S. de Bono is an employee of The Institute of Cancer Research, which has a commercial interest in the development of abiraterone acetate. He has served as a paid consultant for Johnson & Johnson, Medivation, Astellas, Dendreon and AstraZeneca. D. Bianchini has no conflict of interest to declare.

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