Androgen deprivation and immunotherapy for the treatment of prostate cancer

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Abstract

Prostate cancer is the most common newly diagnosed malignancy in men, and the second most common cause of cancer-related death in the United States. The primary treatment for recurrent prostate cancer is androgen deprivation, and this therapy is typically continued lifelong for patients with metastatic prostate cancer. Androgens and androgen deprivation have profound effects on the immune system, a finding that has become more appreciated in an era where immune-based treatments for cancer are being increasingly explored. Preclinical studies suggest that androgen deprivation could potentially positively or negatively affect the use of approved immunotherapies, or those that are being developed for the treatment of prostate cancer. In this review, we provide a brief overview of the different types of androgen deprivation treatments used in the management of prostate cancer, discuss their effects on prostate tumors and the immune system and how they are being explored in combination with immunotherapy. Finally, we address some of the critical questions in the field that must be answered to identify the best approaches to combine androgen deprivation with immunotherapy for the treatment of prostate cancer.

Key Words

- ▶ androgen deprivation
- ▶ immunotherapy
- ▶ vaccine
- ▶ prostate cancer

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Introduction

Prostate cancer is the most common cancer in men, and the second most common cause of cancer death in men in the United States (Siegel *et al.* 2016). Androgen deprivation therapy (ADT), using surgical or chemical castration, is a standard treatment used in all stages of recurrent prostate cancer. Eventually, patients will develop castration-resistant prostate cancer (CRPC) prompting additional therapies, many of which further block the androgen axis. This underscores the importance of androgens and the androgen signaling pathway in disease progression. Although ADT affects prostate tumor cells directly, there is growing evidence that androgens and androgen deprivation also have profound effects on the immune system. Immunotherapy (that involves mobilizing the immune

system to attack tumor cells) has been gaining momentum as an exciting new treatment option. The first FDA-approved immunotherapy for prostate cancer, sipuleucel-T (sip-T), has highlighted the potential use of immunotherapy to improve survival for patients with advanced, metastatic prostate cancer. Other types of immune therapy, notably checkpoint inhibitor therapies targeting CTLA-4, PD-1 and PD-L1, have been approved for the treatment of many other types of cancer and are being explored as treatments for prostate cancer. It has been evident that ADT in and itself has immunomodulatory effects, and given the prevalent usage of ADT as a treatment for progressing and/or metastatic prostate cancer, it is timely to examine how ADT can affect the immune system and potentially

be used with these immunotherapy approaches. In this review, we will explore the immunomodulatory effects of ADT and the rational combinations with immune therapies for prostate cancer.

Role of androgens in prostate cancer

Prostate cancer disease progression is initiated by the formation of prostatic intraepithelial neoplasia (PIN), which can progress to high grade PIN (HG-PIN), followed by adenocarcinoma (as characterized by invasion of the epithelium into the stroma) and metastasis. The prostate is heavily reliant on androgen for growth and function, and treatments targeting production of androgen or the AR itself are used in every stage of prostate cancer disease progression. Metastatic prostate cancer is the lethal phenotype of the disease and is typically treated with chronic androgen deprivation, usually by pharmacological means. Castration induces involution of the prostate and decreases the size of prostatic carcinoma (Huggins *et al.* 1941), which forms the rationale behind androgen deprivation as a therapy. In rodent models, castration induced rapid apoptosis in the rat ventral prostate (Banerjee *et al.* 1995). Androgen deprivation also induces cellular senescence, a stable cell cycle arrest in response to sub-lethal stress.

Although initially highly responsive to this therapy, these cancers almost invariably become resistant and grow in a castration-resistant manner. Historically, CRPC was called hormone-insensitive, or androgen-independent prostate cancer. However, treatment with ADT can induce changes in tumor cells that allow them to grow in androgen-depleted conditions. These changes can include tumors acquiring gene amplifications of the AR (Visakorpi *et al.* 1995), mutations arising in the AR (Eisermann *et al.* 2013), activation of the AR occurring independently of androgen (Lyons *et al.* 2008), tumors upregulation of steroidogenic enzymes (Pfeiffer *et al.* 2011) including *de novo* intratumoral androgen synthesis from androstenedione and DHEA (Cai *et al.* 2011), and persistent occupation of AR on DNA binding sites independent of ligand (Decker *et al.* 2012).

Types of androgen deprivation therapies used in the treatment of prostate cancer

For patients with localized prostate cancer, definitive extirpative therapy includes radical prostatectomy and/or radiation therapy (either external beam or brachytherapy),

delivered with or without adjuvant androgen deprivation. Despite initial success with these treatments, up to one-third of patients will have a biochemical recurrence that is characterized by rising serum PSA (Djavan *et al.* 2003). Patients with biochemical recurrence can then undergo ADT, or remain in surveillance. With evidence of metastatic prostate cancer, patients are treated with androgen deprivation with or without docetaxel chemotherapy (Teo & Scher 2015, Gravis *et al.* 2016), or with or without abiraterone (Fizazi *et al.* 2017, James *et al.* 2017), with the androgen deprivation continued indefinitely. At this stage, ADT can include approaches that decrease serum androgen levels by orchiectomy or by targeting gonadotrophin releasing hormone (e.g. leuprolide, goserelin, degarelix), with or without blockade of the androgen receptor with nonsteroidal anti-androgens (e.g. bicalutamide, flutamide, nilutamide).

Two FDA-approved LHRH agonists include leuprolide and goserelin, both of which are equally effective at shutting down testosterone production (Dias Silva *et al.* 2012). An approved LHRH antagonist that is used in the treatment of prostate cancer is degarelix. These agents work to decrease luteinizing hormone production leading to decreased gonadal production of testosterone. LHRH agonists and antagonists are considered as pharmacological alternatives to surgical castration. Despite substantially lowering serum testosterone, adrenal production of androgens can persist, and it has become clear that prostate tumors can also synthesize androgens. Hence, other therapies are typically added to further suppress androgen levels or signaling. Nonsteroidal anti-androgens include flutamide, bicalutamide, nilutamide, enzalutamide and apalutamide. These agents bind directly to the AR with the goal of blocking AR-directing signaling. Abiraterone is a potent Cyp17A1 inhibitor that results in further decreased testosterone levels by blocking androgen production in non-gonadal tissues. 5 α -reductase converts testosterone to the more potent androgen dihydrotestosterone (DHT), which binds to the AR with greater affinity compared to testosterone. Due to remaining androgenic activity from increased testosterone, 5 α -reductase inhibitors (e.g. finasteride and dutasteride) are not considered as a monotherapy in androgen deprivation treatment for prostate cancer, however, are sometimes used in combination with other therapies.

Many other AR-targeted agents are in development for the treatment of advanced, metastatic CRPC and include androgen receptor degrading agents such as galeterone,

and agents that target the N-terminal domain of the AR such as EPI-506. These androgen pathway targeted therapies are summarized in [Table 1](#). One newer class includes agents (e.g. galeterone and ASC-J9) that lead to degradation of the AR. Galeterone targets the testosterone production by inhibiting Cyp17A1, competitively inhibiting the AR, preventing AR binding to DNA and enhancing degradation of both wild type AR and mutant AR ([Yu et al. 2014](#), [Njar & Brodie 2015](#)). However, phase 3 trials of galeterone were recently halted due to the data monitoring committee determining that galeterone would not show an improvement in patient survival compared to enzalutamide. Another AR degrading compound is ASC-J9, a curcumin analog, which induces AR degradation by disrupting AR association with ARA70, a co-regulator of AR transcription ([Yamashita et al. 2012](#)) and is able to induce degradation of both wild type AR and mutant AR encoding F877L, a mutation conferring resistance to enzalutamide. This agent, although evaluated as a treatment for acne, has not yet been evaluated as a treatment for prostate cancer. Most AR-targeted agents to date have targeted the ligand-binding domain of the AR. However, another class of AR inhibitors targets the N-terminal domain, the domain which interacts with other transcriptional regulatory proteins (e.g. EPI-001) ([Myung et al. 2013](#)). EPI-001 binds to the AF1 domain of the AR, preventing AR transactivation ([Andersen et al. 2010](#), [Myung et al. 2013](#), [De Mol et al. 2016](#)). Furthermore, it induced regression of CRPC xenografts in nude mice with little toxicity effects ([Andersen et al. 2010](#)). A related N-terminal AR inhibitor called EPI-506 is currently being evaluated in phase 1/2 clinical trials (NCT02606123) ([Andersen 2017](#), [Antonarakis et al. 2016](#)).

In summary, ADT results in tumor regression, through the induction of tumor cell apoptosis and senescence.

There are several ways to decrease circulating androgen levels, using either LHRH agonists or antagonists, Cyp17A1 inhibitors or compounds to target the AR itself, such as anti-androgens, AR degrading agents or compounds that use several strategies at once. Invariably, prostate tumors develop mechanisms of resistance to these various methods of ADT.

Effects of androgen deprivation on immune function

Much of the exploration of androgens on immune function came from observed sex differences in immune responses. For example, early studies demonstrated that women produce more IgM and produce more inflammatory cytokines including leptin, IL-1RA, CRP, GM-CSF and IL-5 compared to men ([Butterworth et al. 1967](#), [Furman et al. 2014](#)). When immunized with polyvinylpyrrolidone (PVP), female mice produced significantly more antibodies as measured by antibody titer compared to males, and when male mice were castrated, they produced significantly more antibodies compared to females ([Eidinger & Garrett 1972](#)). Lymphocytes from female mice or castrated male mice were more reactive to alloantigens compared to male lymphocytes ([Weinstein et al. 1984](#)). In addition, autoimmune diseases are more prevalent in women compared to men ([Whitacre 2001](#)). In patients with Klinefelter's syndrome, who carry the XXY sex complement and who are subsequently treated with androgen replacement therapy, there was a significant decrease in antibody production, lymphocyte numbers and CD3+, CD4+ T-cell numbers ([Kocar et al. 2000](#)). Given the sex differences in immunity, it is likely that sex steroids such as testosterone and estrogen can affect immune system function.

Table 1 Summary of the current approved and investigational androgen deprivation therapies for prostate cancer treatment.

Type of ADT	Mechanism of action	Approved/investigational	Examples
LHRH agonist	Persistent LHRH release, leading to pituitary desensitization and subsequent testosterone reduction	Approved	Leuprolide, goserelin
LHRH antagonist	Blocks the LHRH receptor	Approved	Degarelix
CYP17 inhibitor	Blocks a key biosynthetic enzyme in testosterone production	Approved	Abiraterone
AR antagonist	Binds the AR – competitive inhibitor for androgen	Approved	Flutamide, bicalutamide, nilutamide, enzalutamide
5 α -reductase inhibitor	Inhibits 5 α -reductase and prevents converting testosterone to DHT	Approved	Dutasteride, finasteride
AR degrading agent	Targets the AR for degradation	Investigational	Galeterone, ASC-509
N-terminal AR inhibitors	Inhibits the N-terminal domain of the AR	Investigational	EPI-506

Sex steroids have profound effects on the immune system, as summarized in Fig. 1. Testosterone can be converted to DHT by 5 α -reductase or alternatively aromatized to estrogen, and all of which have immunomodulatory effects. The AR is expressed in the thymus in both the epithelium and in the thymocytes (that will differentiate into mature T cells); however, it is AR expression in the thymic epithelium that regulates thymus size (Olsen *et al.* 2001). Administering DHT causes involution of the thymus (Olsen & Kovacs 2001), and conversely castration causes an increase in thymus wet weight (Sutherland *et al.* 2005). The decrease in wet weight of thymus, which is induced by testosterone, is mediated by the Notch ligand Delta-like 4 (Dll4) (Velardi *et al.* 2014).

Androgens have also been implicated in having direct negative effects on T-cell function. Treating CD4+ T cells with either testosterone or DHT increased the immunosuppressive cytokine IL-10 (Liva & Voskuhl 2001). Cytolytic T-cell activity can be suppressed by regulatory CD4+ T cells (Treg) characterized by the expression of CD25+ Foxp3+. Culturing splenic CD4+ T cells in serial dilutions of Leydig cell conditioned media (which produce testosterone), or testosterone directly, resulted in a dose-dependent increase in the number

of CD4+ CD25+Foxp3+ Tregs (Fijak *et al.* 2011, 2015). Furthermore, treating CD4+ T cells with Leydig cell conditioned media significantly increased the production of regulatory T-cell cytokine IL-10 whereas concurrent treatment with the anti-androgen flutamide abolished the production of IL-10, demonstrating that CD4+ CD25+ Foxp3+ Treg function is androgen dependent (Fijak *et al.* 2015). These results were confirmed by another investigator group, and they further showed that an androgen responsive element is located within an intron in Foxp3, suggesting that Foxp3 is regulated by androgen (Walecki *et al.* 2015). Interestingly, increased Tregs in the prostate is associated with reduced PSA recurrence-free survival, and significantly associated with higher tumor grade and higher Ki67 labeling in tumors (Flammiger *et al.* 2013). In summary, the immunosuppressive effects of testosterone may result from several factors, including thymic involution with androgen treatment, decreasing lymphocyte proliferative ability and increased Treg cell expansion and IL-10 expression.

In animal studies, supplementation of androgens may have immunosuppressive activities. Female mice infected with lymphocytic choriomeningitis virus (LCMV) and treated with DHT resulted in a decrease in

Figure 1

Sex steroids have effects on both the prostate and the thymus. Androgens such as testosterone and DHT induces a decrease in thymus weight and an increase in prostate weight. Conversely, androgen deprivation causes an increase in thymus weight and a decrease in prostate weight. Androgens also affect T cells, with testosterone causing an increase in immunosuppressive Tregs and IL-10 production and DHT causing a decrease in IFN γ -secretion by T cells. In addition, estrogen causes an increase in Tregs and increased β 2-microglobulin expression and MHC molecules. Androgen deprivation causes an increase in naïve T cell numbers, increase in antigen-specific T cells and increased IFN γ production as well as causing T-cell infiltration into the prostate.

LCMV-specific T cells and IFN γ production (Lin *et al.* 2010). In an induced mouse model of Grave's disease (an autoimmune disease), mice that were treated with DHT before induction of Grave's disease had significantly lower thyroid hormones compared to controls and significantly lower IFN γ and IL-2 production, suggesting that DHT had an immunosuppressive effect on CD4+ T_H1 T cells (Liu *et al.* 2016).

The observations above suggest that androgens have immunosuppressive effects on CD4+ T_H1 T cells, which further suggests that the removal of androgens by castration should enhance immune function. Castration of aged mice caused an increase in thymus size, and this was due to increased proliferation and decreased apoptosis of thymocytes in castrated aged mice compared to intact aged mice (Sutherland *et al.* 2005). Castration increased absolute levels of T cells in the periphery (Olsen & Kovacs 2011) and also led to increased proliferative response to antigen-specific stimulation compared to sham treatment (Roden *et al.* 2004, Tang *et al.* 2012). Mice treated with the chemotherapy agent cyclophosphamide had significantly fewer lymphocytes, but when they were castrated, they recovered lymphocytes faster compared to intact mice (Roden *et al.* 2004). Male mice infected with LCMV have decreased numbers of LCMV-specific T cells and IFN γ production compared to female mice (Lin *et al.* 2010). However, when male mice were orchidectomized, they had increased numbers of LCMV-specific T cells and increased IFN γ production compared to intact male mice, suggesting that testosterone decreases antigen-specific T cells and that ablating androgens can result in increased LCMV-specific T cells numbers (Lin *et al.* 2010). Treating orchidectomized mice with DHT caused a decrease in LCMV-specific T cells as well as decreased IFN γ production. In other rodent studies, castration caused an infiltration of T cells into the prostate, which were initially T_H1 biased, but later T_H17 biased, and persisted until at least 90 days post castration (Morse & McNeel 2012, Kissick *et al.* 2014). In studies of prostate tumor-bearing mice, castration with immunization similarly elicited a greater number of antigen-specific CD8+ T cells; however, an increase in Tregs was observed with prolonged treatment (Tang *et al.* 2012). When IL-2 was blocked, the increase in regulatory T cells was inhibited, suggesting that IL-2 mediates Treg expansion after castration (Tang *et al.* 2012). Together, all these studies demonstrate that androgen deprivation can enhance the function of T-cell.

Similar findings have been found in human studies. Androgen deprivation led to an increase in the numbers

of circulating naïve T cells and T_H1-biased phenotype shortly after beginning androgen deprivation (Morse & McNeel 2010), and decreased numbers of circulating CD4+ Treg (Page *et al.* 2006). In studies using short-term androgen deprivation prior to prostatectomy, an increase in oligoclonal T-cell infiltration into prostate tissue was observed (Mercader *et al.* 2001).

Manipulating sex hormone levels can also cause testosterone to be aromatized to estrogen, which may also have effects on the immune system. Using steroid binding assays, human CD8+ T cells were determined to express estrogen receptors (Stimson 1988), and subsequent studies using flow cytometry and immunofluorescence identified both ER α and ER β present in CD4+ and CD8+ T cells (Pierdominici *et al.* 2010). In pregnant women, where there is a high estrogen environment, Tregs were significantly increased compared to that in non-pregnant women (Prieto & Rosenstein 2006, Valor *et al.* 2011). Contrarily, female patients with estrogen deficiencies have been observed to have higher absolute CD8+ T-cell counts and lower CD4+ T-cell counts, together suggesting that estrogen may be involved in T-cell homeostasis (Ho *et al.* 1991). Moreover, treatment of prostate cancer cell lines with estrogen has been demonstrated to result in gene changes with upregulation of many genes associated with T-cell recognition, including β 2 microglobulin and MHC molecules (Coleman *et al.* 2006).

In summary, androgens play a role in modulating the immune response. The sexual dimorphism in the immune response is apparent by the heightened production of antibodies and cytolytic T-cell activity in females when compared to males. Furthermore, by manipulating hormone levels through either androgen treatment or by castration, there is evidence that androgens have an immunosuppressive effect. Although castration can increase effector T-cell numbers, there can also be a concomitant increase in the number and function of Tregs. Finally, in the course of treatment with AR antagonists, patients may undergo an increase in estrogen levels due to the aromatization of testosterone, which may also have effects on T-cell homeostasis and recognition of prostate tumor cells by T cells.

Androgen deprivation combined with immune-based treatments – preclinical studies

Several preclinical studies have examined immune-based treatments in combination with androgen deprivation

therapies. In a study conducted by Drake and coworkers, adoptively transferred prostate-specific CD4⁺ T cells largely ignored the native prostate (Drake *et al.* 2005). However, when a tumor was present in the prostate, these transferred CD4⁺ T cells underwent proliferation but were deleted later. However, castration mitigated this tolerance, permitting these cells to expand with effector function following vaccination (Drake *et al.* 2005). They suggested that prostate cancer immunotherapies might be most effectively delivered after androgen deprivation (Drake *et al.* 2005). In contrast to these findings, when mice were vaccinated with DNA encoding prostate stem cell antigen (PSCA), delivered by gene gun and treated with castration either before or after vaccination, mice that were treated with vaccine first followed by castration produced significantly more PSCA-specific interferon gamma secreting T cells compared to castration followed by vaccine (Koh *et al.* 2009). Furthermore, they also observed that androgen ablation caused an increase in dendritic cell maturation and increased expression of costimulatory molecules, and when co-cultured with antigen-experienced T cells, induced higher expression of cytokines such as IL2, IFN γ and TNF α compared to naïve T cells (Koh *et al.* 2009).

Using a Pten knockout prostate cancer mouse model, Akins and coworkers examined the effect of androgen ablation by surgical castration on infiltrating immune cells in the prostate tumor. Similar to the previous study, there was a transient increase in CD8⁺ cytolytic T cells and CD4⁺ helper T cells in the prostate 2 weeks after castration, but by 5 weeks post castration, the numbers of CD4⁺ or CD8⁺ T cells did not differ from pre-castration levels (Akins *et al.* 2010). Furthermore, granzyme B, an effector cytokine of CD8⁺ T cells, was significantly upregulated at 2.5 weeks after castration but decreased again by 10 weeks post castration, indicating that this cytolytic-type T-cell infiltrate was not sustained. The localization of the CD8⁺ T cells was clustered in the stroma, rather than in the neoplastic epithelium. In addition, CD4⁺ CD25⁺ Foxp3⁺ Tregs were significantly increased in the tumor stroma at 2.5 weeks castration, and then throughout both the stroma and epithelium by 10 weeks post castration. Depleting Tregs, and injecting tumor cells expressing the T-cell activating TNF receptor superfamily member LIGHT (CD258) into prostate tumors as an *in situ* vaccination, augmented CD8⁺ T-cell cytolytic activity and significantly reduced prostate tumor weight, suggesting that the combination of androgen ablation, regulatory T-cell depletion and *in situ* vaccination was able to induce an effective anti-tumor response (Akins *et al.* 2010).

Ardiani and coworkers investigated a vaccine using the antigen Twist in combination with the anti-androgen enzalutamide (Ardiani *et al.* 2013). Twist is a basic helix-loop-helix transcription factor that is involved in epithelial-mesenchymal transition and frequently upregulated in metastatic cancer (Ansieau *et al.* 2010, Shiota *et al.* 2015). Twist expression increased as tumors progressed in the TRAMP mouse model, indicating that it is a valid tumor antigen (Ardiani *et al.* 2013). When enzalutamide was administered to male C57/Bl6 mice, there was no change in T-cell function such as proliferation or CD4⁺ effector function, but it did induce a significant decrease in genitourinary tract weight and a significant increase in thymus wet weight (Ardiani *et al.* 2013). Vaccinating male mice with a yeast-based vaccine encoding Twist elicited Twist-specific CD4⁺ T-cell proliferation, and treating male mice with enzalutamide alone also significantly increased Twist-specific CD4⁺ T-cell proliferation compared to control mice (Ardiani *et al.* 2013). However, combining Twist vaccination with enzalutamide significantly increased CD4⁺ T-cell proliferation compared to control or enzalutamide-treated mice. Finally, in the TRAMP model, mice treated with the combination of Twist vaccine + enzalutamide had increased overall survival compared to control mice or mice treated with monotherapy vaccine or enzalutamide alone (Ardiani *et al.* 2013). When Twist was incorporated into a poxviral-based vaccine and administered to age-matched TRAMP mice as a monotherapy, median survival of Twist vaccinated mice was better than control mice (19 weeks vs 4 weeks, $P=0.048$), similar to treatment with enzalutamide vs control (24 weeks vs 4 weeks, $P=0.001$). When the Twist poxvirus vaccine was administered together with enzalutamide, overall survival was significantly better compared to either control mice or mice treated with either vaccine or enzalutamide alone (Kwilas *et al.* 2015). These studies indicate that combining immunotherapy with an anti-androgen can improve the anti-tumor efficacy compared to monotherapy alone. Ardiani and colleagues further demonstrated that enzalutamide and abiraterone together sensitized AR-expressing prostate cancer cell lines to cytotoxic T-cell lysis (Ardiani *et al.* 2014). This sensitivity of CTL lysis was likely mediated by NAIP, an anti-apoptotic gene that was downregulated in LNCaP cells after treatment with either abiraterone or enzalutamide (Ardiani *et al.* 2014).

However, another investigator group found different results, suggesting that AR antagonists may have different effects compared with testosterone depletion when used in combination with immunotherapy.

A preclinical study examined the use of a TLR9 agonist (CpG) as an immunotherapy and the effect of tumor growth in combination with either surgical castration or leuprolide+flutamide (Pu *et al.* 2016). Using a subcutaneous Myc-CaP cell line as a model tumor, when CpG was administered in combination with surgical orchiectomy, there was a synergistic suppression of tumor growth compared to either therapy alone. However, when CpG was administered together with ADT using leuprolide with flutamide, tumor growth was not repressed, suggesting that the immune response was repressed, likely mediated by the AR antagonist flutamide. When OVA-B16 cells were introduced to mice and treated with flutamide, there was a significant decrease in OVA antibody production and OVA-induced IFN γ production, which was not observed in surgically orchidectomized mice. When wild type splenocytes were stimulated with anti-CD3 and anti-CD28 antibody and simultaneously treated with AR antagonists flutamide or enzalutamide, or Cyp17A1 inhibitor abiraterone, or LHRH agonist leuprolide, the AR antagonists significantly decreased IFN γ production of both CD4+ and CD8+ T cells (Pu *et al.* 2016). When CpG was administered before flutamide, the repression of tumor growth was restored, suggesting that AR antagonists inhibit initial T-cell activation. Furthermore, they found that AR antagonist-induced repression of T cells was mediated by an off-target effect of GABA-A receptor inhibition. When CpG treatment was combined with androgen biosynthesis inhibitors such as abiraterone, the combined therapies synergized to induce near complete tumor regression. In summary, these studies demonstrate that ADT can affect the immune response generated during immunotherapy treatment. Furthermore, the type of ADT can either positively or negatively affect the immune system, although this needs to be further investigated in a systematic manner.

Androgen deprivation combined with immune-based treatments – clinical trials for prostate cancer

Cancer immunotherapy was deemed the scientific breakthrough of the year in 2013, predominantly due to the success of T-cell checkpoint inhibitors for the treatment of many different types of cancer (Couzin-Frankel 2013). Despite the limited activity of these agents as monotherapies for prostate cancer, the anti-tumor vaccine Sipuleucel-T (Provenge, Dendreon Corporation) was approved for the treatment of advanced, metastatic

CRPC in 2010. Another anti-tumor vaccine, rilimogene galvacirepvec/rilimogene glafolivec (Prostvac, Bavarian Nordic) is currently in phase 3 clinical trial evaluation. Many immunotherapy agents are also being evaluated, alone or in combination, for patients with prostate cancer. Given the immunomodulatory effects of ADT, it would be rational to combine immunotherapy with ADT to augment the anti-tumor effect. Although many immune-based treatments have been evaluated in patients with advanced prostate cancer, many of whom were concurrently treated with androgen deprivation, fewer studies have specifically explored the combination of ADT with immunotherapy. In this section, we have reviewed the clinical studies that have specifically used ADT in combination with prostate cancer immunotherapies (summarized in Table 2).

As described above, the only current FDA-approved immunotherapy for prostate cancer is sipuleucel-T (sip-T), developed by Dendreon Corporation. Sip-T is an autologous cell vaccine in which peripheral blood is collected from patients, and antigen-presenting cells are activated with PA2024, a fusion protein of PAP antigen fused with GM-CSF. The activated cells are infused back into the patient where it is believed that antigen-specific activated T cells can expand to recognize and lyse tumor cells. Sip-T was approved on the basis of a randomized phase 3 trial demonstrating improved survival of patients with metastatic, CRPC treated compared to control treatment (Kantoff *et al.* 2010). Antigen-specific T-cell proliferation and IFN γ secretion was significantly higher in patients treated with sip-T compared to controls (Kantoff *et al.* 2010, Sheikh *et al.* 2013). Although sip-T was approved for patients already receiving ADT, there have been two studies examining the administration of timing of ADT with sip-T. One study compared the administration of the LHRH agonist leuprolide before sip-T (ADT $\rightarrow$ sip-T) or after sip-T (sip-T $\rightarrow$ ADT) in patients with non-castrate, PSA-recurrent prostate cancer (Antonarakis *et al.* 2017). PA2024-specific humoral responses were significantly elevated following sip-T treatment, although there was no difference between the two arms. T-cell responses were assessed by IFN γ ELISPOT and were similar between the groups at all time points except week 6, where there was significantly higher IFN γ production in sip-T $\rightarrow$ ADT compared to ADT $\rightarrow$ sip-T ($P=0.013$). Furthermore, T-cell proliferation was 2-fold higher in sip-T $\rightarrow$ ADT compared to ADT $\rightarrow$ sip-T (Antonarakis *et al.* 2017). The authors concluded that the timing of ADT might affect the efficacy of immunotherapy, and that a preferred sequence would be to use sip-T prior to ADT in this patient population.

Table 2 Prostate cancer clinical trials specifically using androgen deprivation with immunotherapy.

Immunotherapy	Androgen deprivation	Phase	Rationale/approach	Major finding	Reference
Vaccine: Vaccinia-PSA (Prostvac)	LHRH agonist	Phase I	ADT used to reduce tumor burden prior to vaccination	Safety, feasibility	Sanda et al. (1999)
Vaccine: Vaccinia-PSA (Prostvac)	Nilutamide	Phase II	Patients with castrate-resistant prostate cancer randomized to Prostvac vs. nilutamide with combination at progression	Improved survival observed in patients who received Prostvac prior to nilutamide	Arlen et al. (2005) , Madan et al. (2008)
Vaccine: Sipuleucel-T	LHRH agonist	Phase II	Patients with non-castrate PSA-recurrent prostate cancer randomized to sipuleucel-T followed by ADT or vice versa	Greater immune response observed in patients receiving sipuleucel-T followed by ADT	Antonarakis et al. (2017)
Vaccine: Sipuleucel-T	Abiraterone	Phase II	Patients with mCRPC, already receiving ADT, randomized to receive sipuleucel-T concurrently or sequentially with abiraterone	No differences in immune response observed between study arms	Small et al. (2015)
Vaccine: GVAX	LHRH antagonist (degarelix)	Phase I	Patients with newly diagnosed prostate cancer randomized to degarelix vs degarelix + GVAX vaccine + cyclophosphamide prior to surgery. Goal to determine if ADT + vaccination can increase CD8+ T cell infiltration	(Pending, trial ongoing)	NCT01696877
Vaccine: pTVG-AR	LHRH agonist +/- bicalutamide	Phase I	ADT used to reduce tumor burden, and vaccine targeting AR to target AR-expressing tumor cells as mechanisms of resistance to ADT	(Pending, trial ongoing)	NCT02411786
T-cell checkpoint inhibitor: Tremelimumab	Bicalutamide	Phase I	Patients with non-castrate PSA-recurrent prostate cancer received two 28-day courses of bicalutamide 3 months apart, with anti-CTLA-4 delivered at end of each cycle	Safety, feasibility. Prolongation of PSA doubling time in some patients	McNeel et al. (2012)
T-cell checkpoint inhibitor: Ipilimumab	LHRH agonist + anti-androgen	Phase II	Patients with recurrent prostate cancer randomized to receive ADT +/- ipilimumab	0 of 112 patients without progression at 18 months	NCT00170157
T-cell checkpoint inhibitor: Pembrolizumab	Enzalutamide	Phase II	Patients with mCRPC and progression on enzalutamide received enzalutamide + anti-PD-1	3 of 10 patients with PSA reductions to <0.2 ng/mL	Graff et al. (2016)

Another phase 2 trial examined concurrent abiraterone + prednisone with sip-T treatment vs sequential sip-T → abiraterone + prednisone treatment in patients with metastatic CRPC (notably in patients already being treated with ADT), and assessed the effect on cumulative antigen-presenting cell activation ([Small et al. 2015](#)). In 69 patients treated, there was no statistically significant

difference between the concurrent and sequential arm in terms of antigen-presenting cell activation, or antigen-specific T-cell proliferation and memory T-cell responses ([Small et al. 2015](#)). The study was not powered to examine whether sequential or concurrent administration of sip-T and abiraterone had any effect on clinical parameters such as PSA doubling time or overall survival; however,

the study showed that using abiraterone did not affect the quality of sip-T manufacture.

Another vaccine that is currently undergoing phase 3 clinical trial evaluation for prostate cancer is Prostavac (Bavarian Nordic). Results from a multi-national phase 3 trial (NCT01322490) of Prostavac are expected to be released by the end of 2017. Prostavac-V/TRICOM consists of a vaccinia virus transduced to express PSA and three costimulatory molecules B7.1, ICAM-1 and LFA-3 (TRICOM) which is administered as the initial dose, followed by up to 4 boosts with Prostavac-F/TRICOM, which is a fowlpox virus transduced to express PSA and TRICOM. Preclinical studies with Prostavac in a prostate-specific PSA transgenic mouse demonstrated that the combination of Prostavac with androgen deprivation by means of castration elicited increased T-cell responses, as indicated by increased IFN γ production and PSA-specific T cells (Arredouani *et al.* 2010). A phase 2 clinical study assessed the efficacy of Prostavac, and compared it to the anti-androgen nilutamide (Arlen *et al.* 2005). When PSA progression occurred, patients were offered the combination therapy, adding either nilutamide or vaccine to their treatment regimen. There was no significant difference in decrease in PSA between patients treated with vaccine or nilutamide, and the median time to treatment failure in the vaccine arm was 9.9 months vs 7.6 months in the nilutamide arm. In patients who had nilutamide followed by vaccine, the median time to treatment failure was 5.2 months from the start of combination therapy, whereas in patients who had vaccine followed by nilutamide, the median time to treatment failure was 13.9 months from the start of combination therapy (Arlen *et al.* 2005). A follow-up study after 6.5 years of initiating the therapy showed a trend for increased survival in patients randomized to the vaccine arm with a median time of 5.1 years compared to 3.4 years in the nilutamide arm, although this was not significant ($P=0.13$) (Madan *et al.* 2008). When focused on the subset of patients who went from monotherapy to combination therapy, there was significantly greater survival with the vaccine first followed by nilutamide when compared to nilutamide followed by vaccine ($P=0.045$), suggesting again that vaccine followed by an androgen receptor antagonist may be a preferred sequence of administration.

Two trials have evaluated androgen deprivation as a means to reduce prostate tumor volume prior to the use of an anti-tumor vaccine. In a small phase 1 trial of 6 patients, Sanda and coworkers evaluated Prostavac in patients with early PSA-recurrent prostate cancer after a short course of

androgen deprivation (Sanda *et al.* 1999). More recently, we initiated a phase 1 clinical trial evaluating a DNA vaccine encoding the ligand-binding domain of the AR in patients who had started ADT within 6 months for newly metastatic disease (NCT02411786). This was based on preclinical information demonstrating that vaccination alone, targeting the AR protein itself, could prolong the survival of prostate tumor-bearing TRAMP mice, and that combining vaccination targeting the AR with ADT, could lead to a delay in castration-resistant tumor growth (Olson *et al.* 2013).

ADT can also potentially impact the anti-tumor efficacy of non-vaccine-based immunotherapies, notably immune checkpoint inhibitors. Several immune checkpoints that are currently approved for other cancers include ipilimumab (anti-CTLA4), tremelimumab (anti-CTLA4), pembrolizumab (anti-PD1) and nivolumab (anti-PD1). Clinical trials using immune checkpoint inhibitors alone in patients with metastatic prostate cancer have been disappointing to date (Topalian *et al.* 2012, Kwon *et al.* 2014, Beer *et al.* 2017). A phase 1 clinical trial evaluated the combination of tremelimumab with ADT delivered in pulsatile manner in patients with biochemically recurrent disease. No favorable changes in PSA doubling time were observed shortly after the treatment, however, 3 patients experienced an increase in PSA doubling time several months after completing the treatment (McNeel *et al.* 2012). A randomized phase 2 clinical study specifically evaluated the combination of another CTLA4 inhibitor, ipilimumab, with ADT for patients with recurrent prostate cancer. In that trial, patients were randomized to receive LHRH agonist with an anti-androgen alone or with ipilimumab. None of the 112 patients were progression free at 18 months, the primary endpoint of the trial (NCT00170157). However, in another phase 2 trial combining pembrolizumab with enzalutamide in patients with enzalutamide-resistant disease, 4 of 20 patients achieved a PSA reduction >50%, (Graff *et al.* 2016). Thus, combining ADT with specific checkpoint inhibitors remains an area of interest to potentially increase the anti-tumor effectiveness of these immunotherapies.

Future directions and critical questions to address over the next 5–10 years

The fact that androgen deprivation is the primary treatment for recurrent prostate cancer, combined with its known effects on immune function, make it ideally

suited to be used in combination with existing and emerging immunotherapy treatments for prostate cancer. Notwithstanding, several critical questions remain, which should guide the next 5–10 years of research and clinical trials. First, the optimal ADTs to be used in combination are not really known, as there has been no comprehensive comparison of the various agents described above for their effects on immune function. For example, treatment with LHRH agonists results in a decrease in serum testosterone, with less effect on serum estrogen levels. Monotherapy treatment with androgen receptor agonists such as bicalutamide, however, results in increased serum testosterone and estrogen levels (Di Lorenzo *et al.* 2005). Treatment of prostate cancer cell lines with estrogen has been demonstrated to increase expression of MHC molecules and other molecules associated with immune recognition (Coleman *et al.* 2006). Hence, unopposed action of estrogen from an androgen receptor antagonist treatment, relative to LHRH agonists or agents such as abiraterone that inhibit synthesis of multiple steroid hormones, could have substantially different effects on immune function and recognition. Moreover, results by Pu and coworkers described above suggest that androgen receptor antagonists (flutamide and enzalutamide) can have negative effects on T-cell signaling relative to an LHRH agonist or abiraterone (Pu *et al.* 2016). It is not known whether this is common to all androgen receptor antagonists, but again this is clearly an important area to understand the impact of all of these agents on T-cell function, antigen-presenting cell function and immune recognition of prostate tumor cells.

A second critical question is the appropriate sequence of androgen deprivation with immune-based therapies, and whether this differs depending on the type of immunotherapy approach used. Early clinical trials used androgen deprivation prior to anti-tumor vaccination in an attempt to minimize prostate tumor burden prior to vaccination (Sanda *et al.* 1999). In addition to minimizing tumor burden, androgen deprivation prior to anti-tumor vaccination is rational given its ability to elicit naïve T cells following thymic regrowth that might be primed and activated with vaccination (Morse & McNeel 2010). Animal studies also suggested that androgen deprivation reduced tolerance to prostate-expressed antigens, further suggesting that it might best be employed prior to immunotherapy (Drake *et al.* 2005). However, experimental evidence from other human trials has suggested that anti-tumor vaccination may best be employed prior to androgen deprivation, using androgen deprivation to modulate the response to vaccination, rather than the reverse

(Madan *et al.* 2008, Antonarakis *et al.* 2017). The mechanism for this is not entirely understood, but may be related to changes that occur with persistent androgen deprivation. Studies in mice and rats have demonstrated that initial Th1 biased responses elicited with androgen deprivation diminish over time, and increases in Treg are observed in prostate tissue with longer term androgen deprivation (Akins *et al.* 2010, Morse & McNeel 2012). Hence, immune therapies other than vaccines, perhaps those specifically targeting Treg or other tumor-regulated T cells, might best be used following the start of androgen deprivation. In support of this concept, a recent clinical trial has suggested that patients with advanced prostate cancer may respond to PD-1 blockade therapy when delivered with concurrent enzalutamide, despite having enzalutamide-resistant disease (Graff *et al.* 2016).

Related to this question of optimal sequence of androgen deprivation with immunotherapies is whether androgen deprivation might be better used as an intermittent therapy with immune-based treatments. Given concerns that continuous androgen deprivation might 'select' for castration-resistant disease, many clinical trials have been conducted to compare continuous androgen deprivation vs its use on an intermittent basis for patients with advanced prostate cancer (Alva & Hussain 2014). The largest study powered to evaluate changes in overall survival did not demonstrate a benefit to intermittent therapy, and hence the standard of care has been to continue androgen deprivation lifelong in the setting of metastatic prostate cancer (Sciarra *et al.* 2013). Notwithstanding, early studies in rats have demonstrated that intermittent use of androgen deprivation elicited waves of apoptosis in prostate tissue (Sandford *et al.* 1984). Recent clinical trials have explored the use of intermittent androgen supplementation with androgen deprivation to more rigorously test this approach (Schweizer *et al.* 2016). Other rodent studies have demonstrated that the T-cell infiltrate into prostate tissue observed following short-term androgen deprivation changes over time with prolonged androgen deprivation, notably with a decrease in CD8⁺ T cells and an increase in Treg (Akins *et al.* 2010, Morse & McNeel 2012). It remains unknown whether intermittent use of androgen deprivation might then strategically be used to maintain a CD8⁺ T-cell prostate tumor infiltrate, an approach that may be synergistic with immune-based treatments. One clinical trial has tested this approach using two short courses of bicalutamide and specifically sequenced with CTLA-4 blockade (McNeel *et al.* 2012). Although the study was too small to evaluate efficacy, the approach was found to be feasible.

Finally, a question of major importance is whether the use of androgen deprivation with immunotherapies need be confined to evaluation in prostate cancer or could be explored for other cancers and even other immune conditions. Of note, short-term androgen deprivation has been explored in the setting of stem cell transplantation to help T-cell immune reconstitution (Sutherland *et al.* 2008). Hence, androgen deprivation might be used in other contexts and treatment combinations requiring an increase in naïve T cells. Drake and coworkers have demonstrated in mice that androgen deprivation could reduce tolerance specifically to antigens expressed by prostate tissue, enabling their recognition by subsequent immune-targeted therapy (Drake *et al.* 2005). Certainly the expression of prostate-expressed proteins is affected by androgen deprivation, and this may contribute to changes in the immunogenicity of prostate-expressed genes. Studies using non-prostate tumor models have similarly suggested a benefit combining androgen deprivation with tumor vaccination. However, at present it remains unknown whether the anti-tumor efficacy may be greater to antigens expressed by tissues affected by androgens or androgen deprivation.

In summary, ADT is already a standard treatment for recurrent prostate cancer. Given the systemic immunomodulatory effects of androgen deprivation with increases in thymopoiesis, and the specific effects of ADT on prostate tissue leading to cell death and infiltration by lymphocytes, combining ADT with immunotherapy is a rational direction for improving the efficacy of prostate cancer immunotherapy. Although several clinical trials have explored combination approaches, there remain critical questions related to the best androgen-targeted agents to use for combination approaches, the best sequence of these therapies and whether androgen deprivation might be better used intermittently with immunotherapy approaches. We expect these questions will guide the design of critical murine studies and clinical trials over the next 5–10 years.

Declaration of interest

D G M has ownership interest, has received research support and serves as consultant in Madison Vaccines, Inc. M G has no relevant potential conflicts of interest.

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