Review Article

Immunotherapy in metastatic prostate cancer

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ABSTRACT

Introduction: Prostate cancer remains a challenge as a target for immunological approaches. The approval of the first cell-based immune therapy, Sipuleucel-T for prostate cancer introduced prostate cancer as a solid tumor with the potential to be influenced by the immune system.

Methods: We reviewed articles on immunological management of prostate cancer and challenges that lie ahead for such strategies.

Results: Treatments have focused on the identification of novel cell surface antigens thought to be unique to prostate cancer. These include vaccines against carbohydrate and blood group antigens, xenogeneic and naked DNA vaccines, and pox viruses used as prime-boost or checkpoint inhibitors. No single vaccine construct to date has resulted in a dramatic antitumor effect. The checkpoint inhibitor, anti-CTLA-4 has resulted in several long-term remissions, but phase III trials have not demonstrated an antitumor effect or survival benefit.

Conclusions: Multiple clinical trials suggest that prostate cancer may not be optimally treated by single agent immune therapies and that combination with biologic agents, chemotherapies, or radiation may offer some enhancement of benefit.

Key words: Chimeric antigen receptors, immunotherapy, ipilimumab, prostate cancer, Sipuleucel-T, T cells

INTRODUCTION

The approval of five new treatments for metastatic castrate-resistant prostate cancer (mCRPC) within the last 5 years has been an unprecedented milestone in prostate cancer treatment[1-5] [Figure 1]. Not only have these drugs successfully changed the therapeutic landscape for this disease but at the same time have also introduced new clinical challenges. These challenges include the identification and integration of novel blood-based biomarkers, sequencing or combination of the androgen-receptor (AR) targeting drugs,[3,4] use of early chemotherapy intervention, and more recently, the role of genomic profiling[6] in disease prognosis and treatment. While clinical trials remain the backbone of any drug under development, their role is pivotal towards bringing novel approaches earlier into the clinical arena. The introduction of the first autologous immune-based cellular therapy, Sipuleucel-T[2] has led to a change in the metastatic treatment paradigm; despite a survival benefit, the lack of a robust antitumor effect has made this agent less appealing. How it ultimately fits into the continuum of prostate cancer treatments remains unclear. Overall, there are many challenges that immune therapies bring to the treatment tableau, and it remains unclear whether prostate is in fact an ideal target for such approaches.

EARLY TREATMENT INTERVENTIONS

While the prevailing standard of care for patients with metastatic disease remains hormonal therapy with a GnRH agonist or antagonist, with or without an antiandrogen, those patients who become castration resistant have

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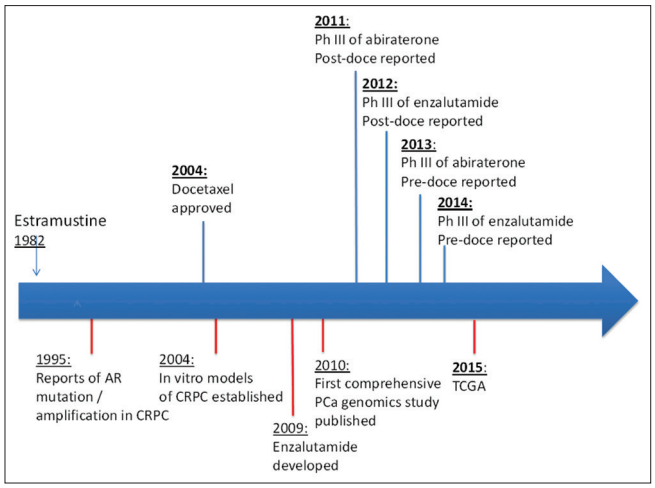

Figure 1: Timeline to prostate cancer drug development

multiple options including the usual attempt at antiandrogen withdrawal.[7] As such, patients could potentially be treated with a second or even third line antiandrogen. The same observations are true for patients currently undergoing treatment with either steroids or the AR targeting drugs. Patients who have failed these modalities could be offered immunologic therapy, clinical trials, or chemotherapy with recent data suggesting the benefits of early intervention with chemotherapy at the time of diagnosis of metastatic disease.[8,9]

The United Kingdom-led STAMPEDE trial[9] confirmed the observations of Sweeney *et al*.[8] and found that adding docetaxel chemotherapy to standard hormone therapy markedly improved survival for men with newly diagnosed advanced prostate cancer not previously treated with hormone therapy (hormone-naive). Men who received docetaxel plus standard hormonal therapy lived on average 10 months longer than those who received only standard therapy. STAMPEDE used a novel trial design that was a multiarm, multistage platform to test whether the addition of treatments at the time of long-term hormone therapy initiation improved overall survival. This is the first time that a clinical trial had multiple arms with different agents in which patients could be studied prospectively. The trial included interim activity analyses based on failure-free survival to select groups to continue accrual for fully powered survival analysis. The patient population included men with metastatic (M1), high-risk localized (N0), or node-positive (N+) prostate cancer who were newly diagnosed or had high-risk recurrent disease following prior localized therapy. These were stratified to receive standard of care, i.e., hormonal therapy ($n = 1184$) or standard of care plus docetaxel at the dose of 75 mg/m^2 ($n = 592$).

The median overall survival was 71 months (interquartile range 32 to not reached) for the hormonal arm-only, 81 months (41 to not reached) for hormones and docetaxel (0·78, 0·66–0·93; $P = 0·006$). These studies endorse the potential benefits of using docetaxel in conjunction with standard hormonal therapy; physician choice based on clinical assessment is still paramount to initiating treatment.

TREATING CASTRATE RESISTANT METASTATIC DISEASE

The standards of care continue to be initiating first and second line antiandrogens i.e., the addition of antiandrogens or AR-directed therapies in the setting of patients who have been on single agent agonist or antagonists or have been on prior antiandrogens. For the latter, a trial of antiandrogen withdrawal is reasonable. It should be noted that following a standard treatment algorithm for all patients may not be reasonable as these patients may have a more aggressive biology and need other means of evaluating the potential of the behavior, i.e., gene profiling of tumor or assessing circulating tumor cells.

When to initiate docetaxel in this setting remains the physician's choice. The rationale for using docetaxel first line after standard hormonal therapies *in lieu* of its immediate use posthormonal therapy may be based on several factors. Patients whose prostate-specific antigen (PSA) is rapidly rising and unresponsive to first-line hormonal therapy are more symptomatic, i.e., failure to thrive, poor oral intake, decreased performance status, or having multiple sites of pain whereby significant radiation would be needed are appropriate candidates to start docetaxel. Not only was a survival benefit observed[10,11] but it also improved the quality of life. Determining the biology through the disease's natural history is important in determining how to proceed with any given therapy.

The approval of enzalutamide[4] (Xtandi™) and abiraterone[3] (Zytiga™) given before or after docetaxel has changed the natural treatment landscape of prostate cancer. However, patients continue to develop resistance to these drugs through a variety of means.[12] As such, new strategies are needed to circumvent resistance and still maintain treatment efficacy.

Patients are aware of the successes of these agents in providing a survival benefit and the rapid onset of antitumor responses. Nevertheless, many patients prefer to avoid the use of "toxins" which they often think are synonymous with chemotherapy and want a more "natural" approach, i.e., using their own immune system to fight the cancer. Many approaches have been used to date including synthetic mimes of known carbohydrate molecules over expressed on prostate cancer cells and cells lines,[13,14] DNA vaccines,[15] prime-boost virus vector vaccines,[16,17] irradiated prostate cancer cell lines transduced with the genes for growth factors,[18] as well as combination approaches with

chemotherapy.[19] We have learned a considerable amount about vaccine trials in prostate cancer [Table 1] but still are behind the accomplishments seen in other solid tumors such as melanoma, bladder, renal cell, and nonsmall cell lung cancers.

Unlike other solid tumors, prostate cancer has been viewed as an inflammatory disease; the extent of immune dysregulation leading to prostate cancer pathogenesis has been focused largely on inflammatory cytokines. Several murine models have been generated in an attempt to understand the transition into malignancy. One novel model of prostatitis showed prostatic mast cell infiltration with subsequent accumulation of neutrophils, T lymphocytes, and macrophages, as well as increased expression of several chemokines. This chronic inflammation preceded the formation of prostatic intraepithelial neoplasia lesions that demonstrated the infiltration by immune cells. Many studies have evaluated the association between specific immune cells and prostate cancer; a majority of these investigated CD3+, CD4+, or CD8 + cells in prostatectomy or biopsy tissue of patients and found such cells to be pro-tumorigenic. For example, a cross-sectional study of lymphocyte infiltration in tumor tissue microarrays from patients with biochemical relapse reported that extremely high or low $CD3^+$ cell counts correlated with reduced PSA recurrence-free survival (RFS).[20,21] CD3 was the only immune cell marker included in this study, and no T-cell subset analyses were performed. While it may be assumed that the variation in clinical outcomes across $CD3^+$ cell quartiles may partially reflect differences in T-cell populations, there are data that suggest that higher numbers of regulatory T-cells (T_{reg}) were associated with more advanced tumor stage and PSA-RFS. This led to the unsubstantiated assumption that the $CD3^+$ cells whose low numbers conferred poor prognoses were protective in nature.[21]

Sipuleucel-T became the first approved treatment for asymptomatic or minimally symptomatic CRPC with a survival benefit.[2] The preparation is comprised of the patient's own peripheral blood mononuclear cells that have been transduced with a gene for a fusion protein of prostatic acid phosphatase and granulocyte/macrophage colony-stimulating factor. The cells are then returned to the patient as three separate infusions each 2 weeks apart. Despite the survival benefit, minimal or no antitumor responses were seen. It has remained unclear as to when a patient would derive benefit. The original trials did not follow patients beyond 6 months; based on data with checkpoint inhibitors in melanoma, the thought is that it would take many months for the treatment to work, preceded initially by a disease flare then a regression. This has not been the case for prostate cancer. However, there appears to be a subtle impact within the immunologic milieu. There is evidence to suggest that antigen-specific T and B cell responses can be generated early, i.e., following the first infusion and these could be res-stimulated subsequently *in vitro*. Cytokines were also associated with T-cell activation and could be detected in the cell culture fluids following the second and third stimulations. Interleukins (ILs) that were detected included IL-2, 4, 5, 6, 10, 13, 17, and interferon gamma (IFN-γ). Tumor necrosis factor-alpha was also induced. There was an increase in known T-cell activation markers CD134 and CD136 on CE4 + and CD8 + T cells after culture with the fusion protein. Recall responses suggestive of sensitivity to the treatment were detected by proliferation responses and IFN-γ production.[22-24]

Table 1: Lessons learned: Prostate cancer vaccine trials

Prostate not an "immunologic solid tumor" compared with melanoma, renal, lung, or bladder cancers
Not significantly hypermutated
Increasing doses of vaccine is not equal to augmentation of immunogenicity, i.e., lower doses likely more immunogenic
Antibodies were generated with specificity for the immunogen but no biologic effect seen
No potentiation of T-cell responses
Immunologic signals (response to therapy) are not immediate; unclear if boosters would be helpful

Many urologists have advised its use in the setting of biochemically relapsed prostate cancer with the consideration that this therapy may be changing the immune milieu early on in the disease thereby making other subsequent therapies more effective. There have been no strong data to support it effects however. Attempts to enhance this approach have been made by others including a combination of Sipuleucel-T with biologic agents.[24-28] These efforts continue in an attempt to optimize the use of Sipuleucel-T within the prostate cancer treatment continuum.

NOVEL CONSTRUCTS WITH PRIME BOOSTS

Despite the enthusiasm for using immune therapies in prostate cancer, there remains a paucity of agents for testing. PROSTVAC, a DNA vaccine comprised a recombinant vaccinia vector as the primary immunotherapy backbone.[16] It is followed by booster immunization using a recombinant fowl pox vector. The vectors contain transgenes for PSA and TRICOM, the latter being 3 co-stimulatory molecules intracellular adhesion molecule-1 (CD54), B7.1 (CD80), and leukocyte function-associated antigen-1 (CD58). Unlike Sipuleucel-T, this construct was based on the inherent immunogenicity of the pox virus. An anti-PSA directed T-cell response is generated but at the same time, other antigens may be exposed that could activate other T cells. This is in part thought to be how Sipuleucel-T works through "antigen spreading". Results of phase I and phase II trials have been encouraging with the phase II trial suggesting a survival benefit comparable to that of Sipuleucel-T. However, the results of the completed phase III trial, are eagerly awaited.

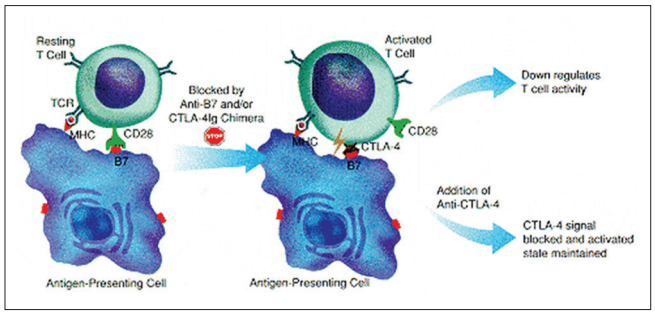

Figure 2: Mechanism of action of T-cell engagement. CD28 signaling promotes T-cell activation and upregulation of CTLA-4 within the cell. Blockade of CD28 signal with CTLA-4 immunoglobulin or B7 antibodies will inhibit T-cell activation. The end result of using antibodies to CTLA-4 allows the inhibition to be released and unrestricted proliferation of T cells. Reproduced by permission of R and D systems

CHECKPOINT INHIBITORS: WHY NOT PROSTATE CANCER?

The excitement over immunotherapy in solid tumors has occurred as a result of the significant and durable responses obtained in several malignancies using checkpoint inhibitors. The first, a monoclonal antibody directed against the checkpoint molecule, CTLA-4[29] (Ipilimumab, Yervoy™) was approved for melanoma in the setting of improved survival and antitumor effects. CTLA-4 is a protein receptor that resides within the T cells and downregulates the immune system [Figure 2]. Upon T-cell engagement with dendritic or antigen presenting cells (APCs), the cells that present cancer or foreign antigens to the T cell, the T cell must have certain costimulatory molecules that tell it to either proliferate or abort its interaction with the APC. Activation of resting or quiescent T-cell requires two complementary signals. Engagement of the T-cell receptor must be accompanied by a second signal that results from the binding of receptors on the T cell with either soluble factors, such as IL-2, or cell-surface molecules on the antigen-presenting cell. CD28 and CTLA-4 are receptors on T cells that play critical roles in the initial activation and subsequent control of cellular immunity. CD28 is expressed constitutively on T cells; it provides a co-stimulatory signal upon binding to target ligands on antigen-presenting cells. Conversely, CTLA-4 is transiently expressed following T-cell activation. The signal delivered through CTLA-4 down regulates T-cell function and inhibits excessive expansion of activated T cells.

A phase I/II dose-escalating trial in patients with mCRPC of ipilimumab alone or following radiation to bone lesions, the latter in an attempt to induce antigen release, demonstrated safety and tolerability of the drug alone and in combination with radiation but predictable autoimmune events such as colitis and hypophysitis occurred.[30] These occurred irrespective of the dose and were treated with high doses of steroids and resolved over time. Several patients sustained durable remissions. The phase III trial of ipilimumab with and without prior radiation for patients who failed docetaxel

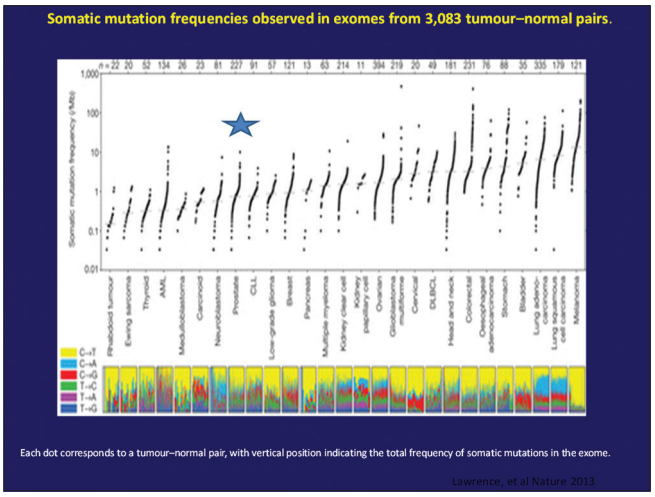

Figure 3: Mutational profiles of solid tumors.[32] Reproduced with permission of publisher

did not confirm an overall survival benefit, but there was a suggestion that patients with visceral metastases had a worse prognosis and poorer survival.[29] Attempts to combine ipilimumab with vaccines have suggested benefit.[31,33]

OTHER STRATEGIES

How do we strategize the implementation of immune therapies when the dramatic impact seen in other solid tumor far outshines that which is seen in prostate cancer? Among the theories for suboptimal responses in prostate cancer is the concern that prostate cancer is not a hypermutated disease as seen in other diseases[34] [Figure 3]. Other approaches now are focusing on "armored" or chimeric antigen receptor directed T cells, whereby the patient's own T cells can be redirected to recognize and kill tumor cells that express a particular antigen on its surface. It has had significant successes in hematologic malignancies such as acute lymphocytic leukemia but has been limited in prostate cancer.[35-38] The reasons are many such as presence of sclerotic, bone disease may prevent these cells from trafficking to sites of active tumor, many more cells may been needed given that the reticuloendothelial system may engulf these cells and prevent them from reaching their target, and the durability of the cells may not be as long as may be needed to effect antitumor effects, i.e., hours to days or weeks.

CONCLUSIONS

While immune therapies can be used to treat at any time during the disease progression, there is still a need for immune agents that can be used either alone or in concert with other therapies. Prostate as a solid tumor remains a challenge in that we are lacking the robust antitumor responses seen with other agents despite survival benefits. It is eagerly anticipated that as we gain more experience

with these agents, we will be able to maximally enhance treatment responses and their durability.

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Conflicts of interest
There are no conflicts of interest.

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