Immunotherapy and Bone-Metastatic Castration-Resistant Prostate Cancer: Hope for the Future

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INTRODUCTION

The immune system is inherently equipped to target and destroy tumors due to the potential development of long-term antitumor memory, which may help avoid recurrence of the disease. The complex relationship between cancer cells and the immune system results in an ability to elude immune destruction. Typically, the antitumor response should result in acute inflammation and tumor elimination but instead, it stimulates chronic inflammation, which promotes tumorigenesis. Therefore, tumors are equipped to 'hijack' normal immune regulation to their advantage, using inflammation mechanisms to promote tumor development, and then at later stages of tumorigenesis promoting a microenvironment of intense immune suppression in order to further inhibitan antitumor reaction [1] (Figure 1).

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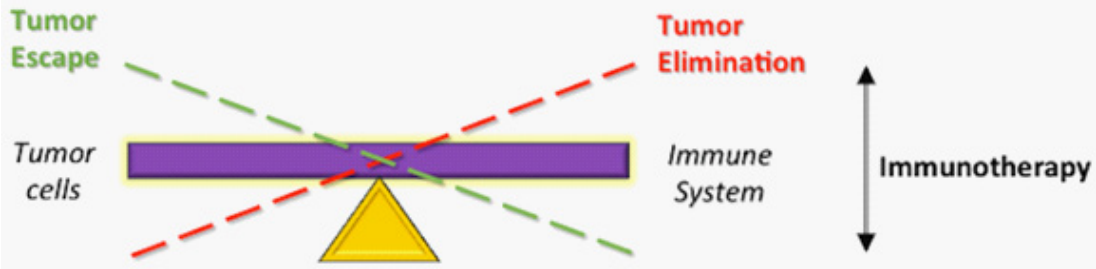


Figure 1: The immune system maintains the equilibrium between tumor elimination and tumor escape or unregulated proliferation. This equilibrium is achieved through immune surveillance, whereby the immune system can specifically identify and eliminate tumor cells on the basis of their expression of specific cell surface antigens. However, in cases where the immune system is not able to completely eliminate the tumor, cancer cells that can resist, avoid, or suppress the antitumor immune response are selected, leading to tumor cell escape from growth regulation. Immunotherapy can restore the balance to modulate antitumor immunity and provide durable and effective tumor growth control.

Prostate cancer epithelial cells typically proliferate in response to sex hormones called androgens, which include testosterone. For this reason, a common treatment option for prostate cancer is to lower the androgen levels in a patient, which can be accomplished by drugs that either stop the testicles from making androgens or act as androgen antagonists. This type of treatment is called hormone therapy or androgen-deprivation therapy **(ADT)**. ADT typically controls both early stage disease progression, when prostate-specific antigen **(PSA)** levels typically begin to rise, and also may help control advanced disease if the tumor remains responsive to ADT. However, in many cases the disease advances towards androgen-independent growth, whereby tumor cells gain the ability togrow in the absence of androgens. This results in a more aggressive form of prostate cancer with limited options for treatment termed‘castration resistant prostate cancer’ or CRPC. For men with this type of disease, additional treatment is needed to help control tumor growth such as chemotherapeutics, which are more aggressive treatment options that in a large majority of cases are not curative and refractory tumors tend to relapse.

Many patients with metastatic castration-resistant prostate cancer **(mCRPC)** experience disease progression during or following the first-line chemotherapy, docetaxel. And recently, novel therapies have emerged that appear to prolong survival in the post-docetaxel setting. These new chemotherapeutics includea novel semisynthetic taxane, cabazitaxel. Cabazitaxel has the ability to overcome taxane resistance and has demonstrated activity in docetaxel-sensitive and docetaxel-resistant cell lines and tumor models [2]. In addition, unlike docetaxel and paclitaxel, cabazitaxel may be effective in patients with spinal cord metastases. This is significant since mCRPC becomes increasingly difficult to treat post-docetaxel relapse, in particular when it spreads to bone tissue (bone-metastatic). We will discuss the current and emerging therapeutics for bone-metastatic CRPC in the following sections.

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THERAPIES FOR BONE-METASTATIC CRPC (MCRPC)

Bone-metastatic CRPC is challenging to treat. The majority of patients are first diagnosed with localized prostate cancer, which can typically be effectively treated with radical prostatectomy or radiation therapy, however, 20–40% of patients develop recurrent disease. Clinical trials involving chemotherapeutic drugs such as docetaxel, for example have demonstrated a mild but tangible extension of CRPC patient survival. Many improvements have been made recently in the therapies available to patients with CRPC, including several new drugs that is either recently approved by the FDA or under clinical trials (Figure 2). However, metastatic CRPC still lacks appropriately effective modalities for its treatment, especially when it spreads to bone tissue, generating skeletal-related events, pain, and significant mortality. Although only 3% of patients have bone involvement at diagnosis, this number rises to 90% in metastatic prostate cancer patients [3-5]. Unfortunately, half of the metastatic cases reach mortality within 30-35 months from diagnosis [6-8]. The types of bone lesions associated with prostate cancer include the increased formation of immature non-mineralized bone by osteoblasts [9], and increased bone resorption by osteoclasts [10-13]. The disrupted balance between osteoblasts and osteoclasts leads to fractures and severe pain in patients. Under normal conditions, osteoclasts resorb bone whereas osteoblasts replace the void with new bone. However, cancer metastases disrupt this process to favor bone destruction and enhance formation of immature non-mineralized woven bone [14]. Crosstalk between osteoblasts, osteoclasts and tumor cells augments both bone and tumor lesions, creating a pathological cycle that feeds tumor growth. Particularly vital molecules for furthering tumor growth in the bone include IL-6 and IL-11,which act as critical players for all three signaling axes (TNF, IL1β, RANKL)[15-26], but other important axes for tumor-bone communication also involve TGFβ signaling [27], which acts to impact both bone and tumor cells to augment the malignant crosstalk [28-29]. Therefore, there is a large demand for treating CRPC and also the bone tissue involved in the malignant crosstalk with the tumor cells. Additional treatment strategies are needed that might (i) treat and prevent CRPC recurrence or (ii) stabilize skeletal-related events and/or repair bone lesions, thus increasing patient survival.

Recently developed or FDA-approved CRPC therapies have some promising effects on either tumor or bone cells. The most promising therapies for combination are therapies to reduce tumor: bone communication and cyto reductive therapies. Therapies for disrupting tumor: bone communication and reducing bone resorption include zoledronic acid (bisphosphonate) and desonumab (receptor activator of nuclear factor κB **(RANK)** ligand monoclonal antibody). These agents can delay time to skeletal-related events **(SRE)** but cannot stimulate mature bone and do not effectively inhibit tumor growth. Radium-223 is a promising bone-seeking radioisotope that extends survival by 3 months and delays SRE by 5 months, yet bone density is not restored. Also in development are compounds that inhibit bone resorption (new RANKL monoclonal antibodies), inhibit cancer cell migration (SRC kinase inhibitors), target the bone matrix (MMP or TGFβ pathway inhibitors), stimulate bone formation (endothelin A receptor inhibitors), and those

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that target tumor and bone such as IL-6 monoclonal antibodies [30]. Despite promising results for these agents, common challenges still include a lack of impact on overall survival, modest effects on bone turnover, and/or antitumor responses. Cytoreductive therapies for metastatic prostate cancer include local-field external-beam radiation, androgen deprivation therapies (abiraterone or enzalutamide), and taxanes (docetaxel and cabazitaxel). One critical problem for treating metastatic CRPC is that tumors frequently relapse following the first-line chemotherapy (docetaxel). The second-generation taxane cabazitaxel extends survival by 3 months and can treat docetaxel relapse, yet there are no reported effects on restoring bone. Endocrine disruptors enzalutamide and abiraterone both can reduce tumor burden and increase survival by 4-5 months. Unfortunately these drugs only delay the time to SRE ultimately resulting in a large proportion of tumors relapsing soon after treatments. There are however a range of emerging compounds including the VEGFR2/MET/KIT inhibitor Cabozantinib which have shown impressive preclinical effects on restoring bone [31]. Unfortunately, preliminary (unpublished) reports indicate it failed to improve survival in Phase III clinical trials. Despite considerable success, current therapies only prolong median survival and/or delay time to SRE by a few additional months. Because no agent can restore bone and residual tumor cells can evade antitumor agents by alternative survival mechanisms there remains a critical need for an intervention that can significantly impact tumor growth rate in order to decrease tumor burden while simultaneously restoring bone. Immune therapy has high potential for meeting these multifunctional needs by using cytokines that induce antitumor and pro-osteogenic effects.

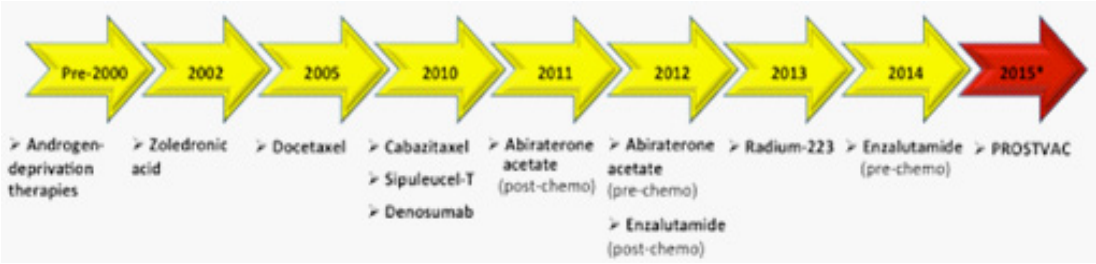


Figure 2: Current approaches for treating metastatic CRPC. Several approaches have been developed since pre-2000 for metastatic CRPC and these are discussed in the text.

Immunotherapy as a Tool for Synergizing with Current CRPC Therapies

Immunotherapy, or the concept of boosting the immune system to target and destroy cancer cells, has been a goal of cancer treatment for over 100 years. However, limited success has been achieved with traditional immunotherapy, as cancer cells tend to evolve mechanisms that evade immune detection. Research trials have shown that immunotherapy may represent a nontoxic and viable approach for stimulating both immunologic and clinical responses in men with prostate cancer [32]. A promising tool to completely eliminate CRPC may beto utilize immunotherapy to inducea robust immune response [33]. Immunotherapy may enhance natural

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immune surveillance and may be particularly effective when combined with biological or chemical therapies to reduce tumor bulk (cytoreductive). A key challenge, however, is how to best integrate the most promising novel therapy modalities into the current clinical space to enhance the efficacy of current drugs while maintaining low toxicity to patients [34]. A wide array of gene therapy techniques is being used to overcome this limitation and utilizing also cell-based approaches to deliver immunotherapy. Current immunotherapy approaches or those in development will enable change in how prostate cancer is treated. The current and future combination therapy approaches will be described in the following sections.

PERFORMANCE OF CURRENTLYUSED IMMUNOTHERAPY FOR CRPC (MONOTHERAPY OR SINGLE-AGENTS)

There are several types of immunotherapies currently studied in CRPC. These include cancer vaccines (autologous, cell-based, virus-based, and DNA-based), other cell-based therapies (adoptive transfer of T cells and mesenchymal stem cell delivery of secreted factors or cytokines), immune checkpoint inhibitors, and gene delivery-based immune therapies (delivery of cytokines expressed from nonviral or viral vectors).

Cancer Vaccines

Provenge or sipuleucel

T. Cancer vaccines signify an evolution in CRPC treatment from conventional chemotherapeutic regimens to immunotherapies that are useful in providing single or multiple tumor antigens to the immune system. An efficient cytotoxic immune response against a tumor involves collaboration between several immune cell types in the adaptive and innate immune system. Cancer vaccines are not made to and cannot prevent disease, contrasting from vaccines for infectious agents. They are instead meant to eradicate or at least impede cancer cells from spreading, partly by granting the diseased cells with antigenic and highly immune stimulatory cellular debris from autologous cells. These autologous cells – which are harvested from the patient beforehand – are subsequently grown *in vitro* (established cancer cell lines can be used instead of autologous cells) where they are engineered by adding one or more genes. These are often cytokine genes that can generate pro-inflammatory immune stimulating molecules, or highly antigenic protein genes, introduced into the autologous cells so that these antigens can be presented to the immune system at a higher rate. The altered cells are then allowed to grow *in vitro* only to be harvested so that the cellular contents can be integrated into a vaccine [35].

For example, autologous dendritic cells can be used to deliver prostate cancer antigens for successful *in vivo* T cell activation. Dendritic cells are resourceful antigen-presenting cells that launch an antigen-specific immune response through uptake, processing, and presentation of antigens to T cells. For example, the only commercially available FDA-approved vaccine, Sipuleucel-T, is a dendritic cell vaccine that induces immunity against cancer, prolonging overall

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survival (Figure 3). Generally, in such procedures, immune cells are taken from the patient and incubatedex vivo (outside the body) with a fusion protein in order to elicit an immune response against tumor-specific or tumor-associated antigens (in this case, a fusion protein composed of prostatic acid phosphatase and granulocyte-macrophage colony stimulating factor). Once these hyper-responsive cells are injected back into the patient, they start attacking PAP-expressing prostate cancer cells. Interestingly, sipuleucel-T appears to provide a greater treatment benefit when patients have more favorable baseline prognostic factors, i.e. earlier or lower grade disease [36]. This suggests that immunotherapy use should be included as early as possible in any combination regimen for treating advanced CRPC.

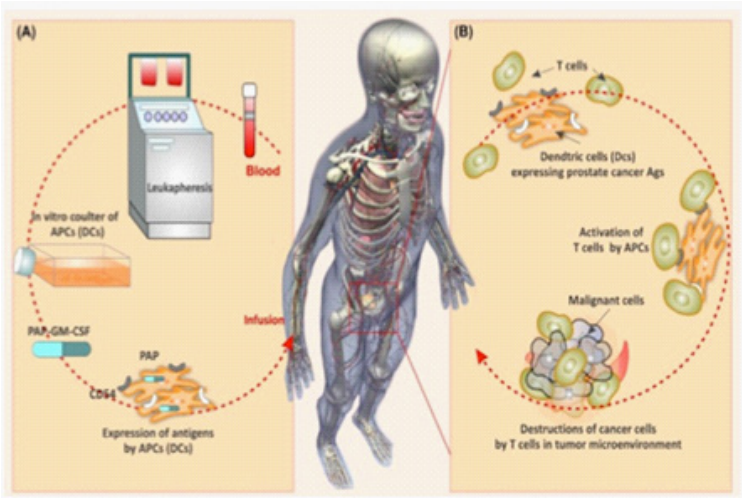


Figure 3: Schematic representation for the cell-based sipuleucel-T immunotherapy for prostate cancer. (A) Procedure for harvesting and processing cells for this cell-based treatment modality. (B) Sipuleucel-T or Provenge™ production begins with the isolation of dendritic cells **(DCs)** as antigen presenting cells **(APCs)** from the patient. Then, after *in vitro* propagation of the DCs in the presence of a fusion protein composed of prostate acid phosphatase **(PAP)** and granulocyte–macrophage colony-stimulating factor **(GM–CSF)** as an immune responses enhancer, the reprogrammed DCs expressing CD54 and PAP (panel A) are re-infused into the patient to activate a T cell response against the prostate tumor (panel B). *Reproduced from [37] under the terms of the Creative Commons Attribution License (http://creativecommons.org/licenses/by-nc/4.0/).*

Prostvac

Another example of an anti-cancer vaccine is Prostvac, which uses vaccinia and fowlpox viruses to deliver prostate-specific antigen **(PSA)** along with three costimulatory molecules (B7.1, ICAM-1, and LFA-3) directly to cancer cells. The virus vectors fuel an immune response against the PSA antigen, which directs the immune system to attack prostate cancer cells. Viral vector-based vaccines such as Prostvac mimic natural infection and thus can induce a potent immune

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response. A phase II study with minimally symptomatic metastatic CRPC patients showed an 8.5 month survival advantage with Prostvac compared to controls. At a follow-up of 3 years, >30% of patients were alive compared to 17.5% of controls [38]. Prostvac is being applied with or without granulocyte-macrophage colony stimulating factor **(GM-CSF)** in a phase III trial of asymptomatic or minimally symptomatic metastatic CRPC patients (NCT01322490) [39], originated in November 2011. GM-CSF is a potent immune stimulatory cytokine. The results of this trial are expected by late 2016 or early 2017.

Gvax

This is a cell-based vaccine derived from an irradiated mixture of two prostate cancer cell lines, LNCaP and PC3, modified to secrete GM-Coffin Phase I/II trials, Gvax appeared to be well tolerated with both high ($3x10^8$ cells) or low dose boosts ($1x10^8$ cells) and median overall survival was 8 months higher in the high dose boost group as compared to radiotherapy alone or low dose boost [40]. A subsequent Phase I/II study with mCRPC confirmed that Gvax was generally safe and that high dose boosts were most effective at extending patient survival [41]. These promising phase I/II trial results led to the initiation of two phase III studies, which were terminated early either due to a lack of efficacy as compared to docetaxel/prednisone therapy or a >42% increase in mortality (reviewed in [42]). According to some experts in the field, these two trials were problematic since the optimal dosing of docetaxel for combinatorial regimens had not yet been determined in one and since a placebo control group was not used in the other one. These results suggest that Gvax may not have a favorable profile as a monotherapy for CRPC, but it continues to be explored in combination regimens in 9 clinical trials for other tumor types such as pancreatic and colon cancer (NCT02243371, NCT01966289) [39].

Cell-Based Therapies

T cell adoptive transfer

T cells and antibodies are the most potent constituents of a specific immune response against tumors. Among them, CD8+ cytotoxic T lymphocytes **(CTLs)** efficiently destroy tumor cells and CD4+ T cells increase the ability to present antigens that are innate to dendritic cells, while supporting the stimulation of tumor-reactive CTLs. Chimeric antigen receptor **(CAR)**-engineered T cells hold great promise as combination therapies that can enhance the current modalities for mCRPC. This is because the prostate expresses many unique tissue-specific proteins or antigens and CAR T cells can be used to target these antigens. Preclinical studies have shown the ability of prostate-specific membrane antigen **(PSMA)** CAR T cells to proliferate and recognize PSMA+ targets both *in vitro* and in animal models. But, although promising, clinical translation of CAR T cells will require a better understanding of how to enhance several aspects of their accessibility to bone metastases of CRPC.

For example, the migration and infiltration of CAR T cells can be a main challenge to this therapy, since the prostate cancer bone metastases microenvironment is strongly immune

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suppressive [43]. Further, inclusion of homing or chemokine receptors may improve T cell recruitment to bone metastases, as may antibody-based combination therapies to normalize the tumor vasculature (Figure 4). Introduction of multiple stimulatory domains also might help CAR T cells to overcome inhibitory signals from the tumor microenvironment. Osteoclasts at the bone-metastatic site also produce immunosuppressive transforming growth factor **(TGF)**-β. The constant activity of osteoblasts and osteoclasts severely remodels the microenvironment and hinders T cell function. Blocking osteolysis thus may help T cell trafficking, and engineering dominant-negative TGF-β receptors into CAR T cells may improve their function. The inclusion of inducible interleukin **(IL)**-12 in CAR T cells creates a better environment for the T cells to work in and can activate bystander immunity to kill antigen-negative tumor cells. Local secretion of IL-12 can recruit other effector immune cells, such as macrophages and neutrophils, to target antigen-negative tumor cells and tumor stroma. Combination with radiotherapy or androgen deprivation therapy also likely will enhance effectiveness of CAR T cell therapy, since these therapies can act as immunomodulatory agents, inducing antigen release and activation of bystander immunity. Because of the highly immunosuppressive environment present in bone metastases of CRPC, CAR T cells need sufficient co-stimulation; therefore combination therapy is likely to be the most promising setting for their clinical application.

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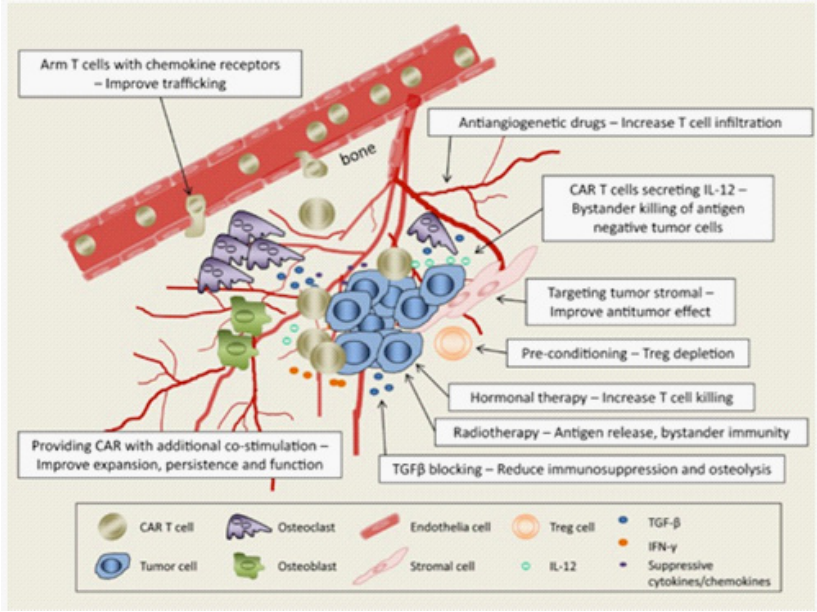


Figure 4: The tumor microenvironment in prostate cancer bone metastases and challenges for chimeric antigen receptor **(CAR)** T cell therapy. Efficacy of CAR T cell therapy may be enhanced by combination with other therapies that might either improve vessel quality, target T cells to tumor vasculature antigens, or reduce immunosuppression by transforming growth factor **(TGF)-β**. Also, other cell types can be modulated in the tumor to reduce sources of inhibitory cytokines, and these cells include regulatory T cells (Tregs) and myeloid-derived suppressor cells **(MDSC).** The inclusion of interleukin expression by CAR T cells such as IL-12 may enhance their ability to activate bystander immunity and promote killing of antigen-negative tumor cells. Radiotherapy can induce antigen release and activate bystander immunity, while androgen deprivation therapy can render tumor cells more sensitive to T cell killing via immunomodulation. *Reproduced from [43] under the terms of the Creative Commons Attribution License (http://creativecommons.org/licenses/by-nc/4.0/).*

Other Cell-Based Immune Stimulating Therapies

Mesenchymal stem cells may serve as vehicles to deliver cytokine immunotherapy

Another emerging cell type that can be used to deliver therapeutics to CRPC is mesenchymal stem cells **(MSC)** and this topic is reviewed in [44]. Examples of MSC used in delivering therapeutics include a cell population called adipose-derived stem/stromal mesenchymal cells **(ASC).** ASC can be useful to cancer therapy since, like CAR T cells, they also can migrate towards a chemokine gradient generated by tumor cells, and are particularly suited to influence the immune response within tumor microenvironments. Depending on several critical variables including the quantity of ASC utilized, these cells can either elicit an immunosuppressive phenotype or a pro-inflammatory phenotype. Therefore, their emerging use requires caution and extensive preclinical testing prior

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to translation in the context of mCRPC. However, their preclinical use so far suggests that gene-modified ASC might be of special promise for treating aggressive tumors including melanoma and prostate cancer. For example, a preclinical study has examined the promise of a nonviral DNA integrating vector for stable transgene expression to deliver pro-apoptotic genes. ASC were modified to express PhiC31 recombinase and/or piggyBac transposase and either express Tumor Necrosis Factor (Ligand) Super family, Member 10 **(TRAIL)**, Interferon gamma **(IFNγ)** or TRAIL+IFNγ in mouse models of melanoma [45]. Although the rationale was that IFNγ might sensitize cancer cells to TRAIL-induced apoptosis by up regulating Caspase-8 through a pathway dependent on STAT1/Interferon Regulatory Factor 1, ASC expressing both genes did not appear to have more potent effects as compared to IFNγ alone. Enhanced green fluorescent protein **(EGFP)**-labeled ASC was detected at the periphery of the tumors following intravenous injection at a ~0.5% abundance relative to tumor cells *in vivo*. The mechanism was recruitment of $CD8^+$ T lymphocytes by the IFNγ expressing ASC, while cells expressing T-Cell Surface Glycoprotein CD4 **($CD4^+$)** and Interleukin-2 **($IL2^+$)** remained constant in the tumor environment of ASC-IFNγ treated mice. Thus it appears that gene-modified ASC, in particular with IFNγ, are a promising modality that may enable tumor recruitment of effector T cells expressing T-Cell Surface GlycoproteinCD8 **($CD8^+$)** and reduce tumor-promoting Treg, with a likely effect of reducing tumor burden.

For advanced prostate cancer, a preclinical study suggested that expression of anti-angiogenic and anti-inflammatory agent pigment-epithelium derived factor **(PEDF)** from ASC was more potent than expression of IL-24/MDA-7 (Interleukin-24/melanoma differentiation-associated gene 7). ASC-PEDF completely prevented prostate adenocarcinoma **(PC3)** xenograft tumor formation, even in *nu/nu* immune-compromised mice, which lack thymic function [46,47]. On the other hand, ASC-MDA7 could not inhibit growth of prostate adenocarcinoma cells unless the ASC were present within an immune competent host. Thus the potential immune-promoting activity of the IL-24 gene may be associated with immune stimulatory effects, and activity has been reported for IL-24 in immune stimulation **(Th1)**, as well as IL-20-receptor mediated cytotoxicity [48]. We predict that further study of the safety of these cellular therapeutic modalities can yield promising translation of ASC interventions for cancer treatment. There are nearly 200 clinical trials active on *clinicaltrials.gov* on the use of ASC for a wide variety of regenerative purposes and some of these are related to cancer therapy. An active trial aims to examine whether ASC can improve urinary incontinence in patients undergoing radical prostatectomy (NCT01799694) [39], as well as to provide data on how safe the localized administration of these cells can be for prostate cancer patients. This and emerging studies may reveal important aspects regarding the safety of ASC in the context of localized or metastatic CRPC.

Immune Checkpoint Inhibitors alter the Tumor Microenviroment

The term “immunecheckpoint” refers to the molecules and pathways that serve as checks and balances in the regulation of the immune system. A number of checkpoints limit the immune response of the host to progression of mCRPC, one of which is an immune suppressive and

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pro-angiogenic cell called the myeloid-derived suppressor cell **(MDSC)**. Tasquinimod is a small molecular oral inhibitor of S100A9, a key cell surface regulator of MDSC function, and has shown anti-angiogenic, antitumor and immune-modulatory properties in preclinical models of prostate cancer and other solid tumors. A large randomized phase II trial of tasquinimod in men with chemotherapy-naïve minimally symptomatic mCRPC showed prolonged progression-free survival by radiographic and symptomatic measures (69%) as compared with placebo (37%), which was also associated with improvements in overall survival. Tasquinimod was examined in a global Phase III randomized trial in men with bone mCRPC and, while it significantly improved radiographic progression-free survival, this did not result in an overall survival benefit. Favorable effects included improvement in alkaline phosphatase and lactate dehydrogenase levels over time, suggesting that tasquinimod might delay tumor progression in bone. Since it is a promising therapy [49], tasquinimod is under evaluation as a combination therapy inprostate cancer and as a single agent in other solid tumors. The efficacy of tasquinimod in delaying metastatic progression over time in clinical trials appears to be related to diverse mechanisms of action, including inhibition of the hypoxia-induced angiogenic switch through epigenetic regulation of HDAC3/4 interactions. These mechanisms enhance the odds for tasquinimod success compared to previously unsuccessful anti-VEGF therapies in men with mCRPC. A large Phase III trial has been completed recently which evaluated tasquinimod in men with asymptomatic or mildly symptomatic mCRPC (NCT01234311) [39], with results expected in 2016.A current trial is examining Taquinimod with Cabazitaxel for treating docetaxel-relapsed mCRPC (NCT01513733). These and other large, controlled trials will provide critical information on whether Tasquinimod impacts overall survival in order to validate its role as a standard agent or in combination with other therapeutics for men with mCRPC.

Another immune checkpoint inhibitor is Ipilimumab, ananti-cytotoxic T lymphocyte associated antigen 4 **(CTLA-4)** monoclonal antibody. CTLA-4 is an inhibitory receptor that turns off the immune response by downregulating pathways involved in T cell activation, including reduced T-cell receptor-dependent activation of the mitogen activated protein **(MAP)** kinases ERK (extracellular signal regulated kinase) and JNK (Jun N terminal kinase), as well as of the transcription factors nuclear factor k-B **(NF-kB)**, AP-1 and NF-AT (nuclear factor of activated T cells), resulting in decreased cytokine production by T cells and cell cycle arrest. Ipilimumab targets CTLA-4 to turn the immune response back on and augment T cell mediated immune responses [50]. Ipilimumab **(IPI)** became the first checkpoint inhibitor to receive FDA approval, based on demonstrated overall survival benefit in patients with unresectable or metastatic melanoma in phase II trials. IPI has shown some promise in a multicenter Phase I/II trial with or without radiotherapy, whereby PSA levels declined in 25% of patients treated with IPI alone versus 12% in IPI plus radiotherapy [51]. And although there were several adverse effects reported involving skin, gastrointestinal tract and liver, IPI did not appear to potentiate radiotherapy-associated toxicity. In the Phase III studies that followed, IPI was evaluated as a single agent in mCRPC patients that had relapsed post-docetaxel [52], but this study did not meet its primary

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endpoint of overall survival compared to placebo. The adverse events reported included diarrhea, fatigue, anemia, and four IPI-related deaths. An important aspect of this study was that patients had visceral metastases; a prognostic factor associated with end-stage mCRPC likely to adversely affect overall survival. IPI appears to have activity in patients with better prognostic factors, i.e. lacking visceral metastases, which suggests further investigation of IPI might be successful in patients with favorable prognostic features and in combination with other therapies. The second Phase III trial with IPI might give better might result in more positive therapeutic effects, since IPI was utilized in the context of asymptomatic or minimally symptomatic mCRPC patients with a lower tumor burden [53]. Results are anticipated in 2016.

Interleukin-24 or MDA-7

Another promising approach for treating mCRPC is to explore gene delivery of cytokine IL-24 or MDA-7. Delivery of viruses expressing IL-24 appears to be particularly promising and can be enhanced when administered in combination with microscopic gas bubbles carrying a chemotherapeutic agent. In a recent study, viruses that conditionally kill tumor cells (and not normal cells) are used which are called conditionally replication-competent oncolytic adenoviruses or cancer terminator viruses **(CTV)**. CTV have several advantages as a gene therapy approach since they can cause oncolysis of the targeted cell as well as bystander tumor cells while sparing normal cells. Also, during this process, the virus spreads within a tumor to reach and lyse a larger number of cells than conventional viral vectors. Therefore, CTV are currently considered a very attractive tactic to treat various cancers. However, since prostate cancer proliferates relatively slowly, it may require repeated gene therapy applications over the life span of the patient to be effective. In this context, CTV that can selectively induce cytolysis in prostate cancer cells along with producing a therapeutic gene that can recruit immune cells to the tumor could represent an effective treatment for bone-metastatic disease.

The therapeutic potential of CTV has been evaluated in human clinical trials [24], where they have been found to be safe and well tolerated in patients with advanced cancers, although therapeutic efficacy as single agents was not promising. A potential explanation to the efficacy challenge might lie in the route of CTV administration (intratumoral), which could suffer from limited spread from the injection site. To the best of our knowledge, although MDA-7/IL-24 (Ad-MDA7, INGN 241) has proven efficacious in preclinical studies and also in a Phase I/II clinical trial with multiple advanced cancers [54], CTV expressing MDA-7 has not yet been used clinically for mCRPC. Future studies likely will examine the efficacy of CTV expressing cytotoxic or cytokine genes in the context of combination therapy.

EMERGING COMBINATION THERAPIES FOR CRPC

Anti-androgens might be Promising for Combination Therapy as they are Immunomodulatory

Despite its promise, all modalities of immune therapy appear to still benefit from 'priming'

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strategies in order to more efficiently promote tumor cell elimination by the immune system. It is likely that the optimal clinical space for immunotherapy will be in the context of combination regimens, either with chemotherapy or radiotherapy (Figure 5). The main reason for priming tumor cells prior to boosting the immune response is that although tumor cells resist direct physical effects of chemotherapy, they respond to certain chemo modulatory effects that enhance cytotoxic T lymphocyte **(CTL)** killing. One of these mechanisms is immunogenic modulation **(IMO)**, which appears to occur at sub-lethal doses (Figure 6a). IMO is known to develop following tumor cell exposure to low-doses of chemotherapy, with alterations in tumor cell phenotype that prime cells for elimination by the immune system. The mechanism underlying IMO appears to involve triggering of calreticulin translocation to the surface of apoptotic cancer cells, which in turn allows the uptake of such dying cells by dendritic cells through scavenger receptors and enable presentation of tumor-associated antigens or immunogenic markers. These dendritic cells activate naïve T cells, which ultimately infiltrate the tumor forming stable immunological synapses, facilitating the killing of tumor cells by CTLs. IMO is a useful tool for cancer therapy since it is common for tumor cells to evade death during or after treatment. And although many different chemotherapeutic drugs modulate antitumor immune responses, induction of IMO appears to be restricted to only a few chemotherapy agents. One example is illustrated in Figure 6b, where highly aggressive prostate adenocarcinoma cells can be stimulated to express a variety of immunogenic markers [55]. Interestingly, the extent of immunogenic marker expression varies between the different classes of chemotherapeutics, suggesting some treatments are likely to be more effective for priming tumor cells for elimination by the immune system.

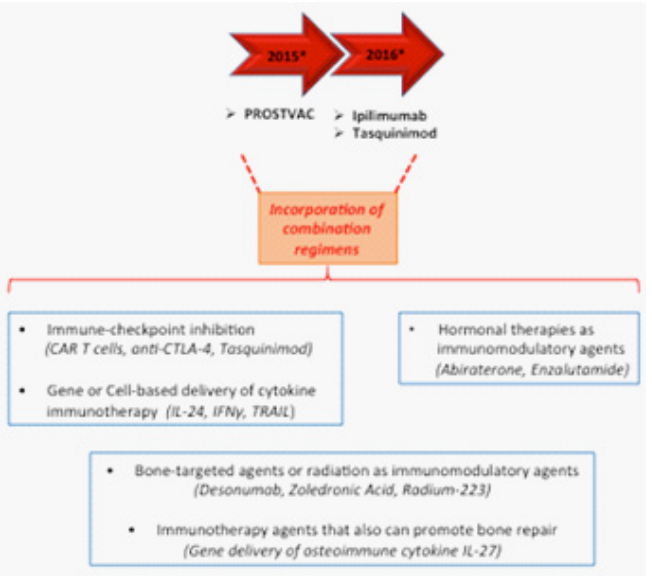


Figure 5: Potential for sequencing immunotherapy in CRPC. Evolution of therapies over time and current promising combinations that are introducing immunotherapy into the clinical space for mCRPC.

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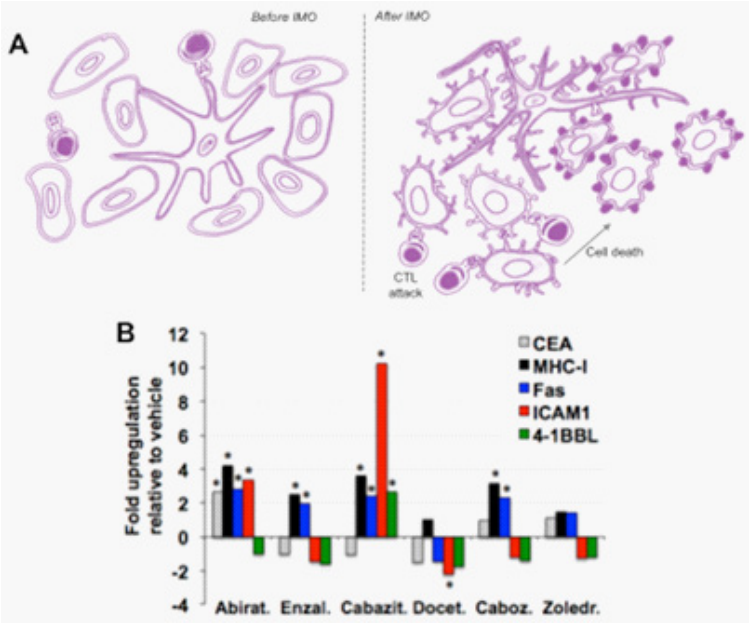


Figure 6: Immunogenic modulation may aid in sequencing cell based, gene based or other immune therapy.(A)Immunogenic modulation **(IMO)** is a powerful mechanism to augment combination therapies by promoting tumor cell exposure to nonlethal cellular doses of chemotherapy, altering the phenotype of the tumor microenvironment and thus enhancing cytotoxic T lymphocyte **(CTL)** cell-mediated tumor-cell killing. (B) Our data suggests that the most effective IMO inducing agents appear to include cabazitaxel, abiraterone, followed by enzalutamide and cabozantinib. TRAMP-C2Ras cells treated with sublethal cellular chemotherapy doses for 24h provides a model of residual cells (qPCR; *,$p<0.04$ vs. ctrl). **CTL:** Cytotoxic T lymphocyte; **CEA:** Carcinoembryonic antigen; **MHC-I:** Major histocompatibility complex class I; **Fas:** First apoptotic signal; **ICAM1:** Intercellular adhesion molecule 1; **4-1BBL:** 4-1BB ligand or CD137L, a type II glycoprotein belonging to the tumor necrosis factor super family; Abirat., Abiraterone; Enzal, Enzalutamide; Cabazit, Cabazitaxel; Docet, Docetaxel; Caboz, Cabozantinib; Zoledr, Zoledronic Acid.

Bone-metastatic CRPC are good targets for IMO combination therapy since they are very difficult to treat due to an altered balance between bone resorption and bone formation that enhances tumor growth and promotes severe pain and fractures at multiple sites [56-57]. Therefore, the boost provided by IMO in combination with immune therapy can break the immunesuppression cycle at tumor bone metastatic sites to help eliminate tumors. Furthermore, some immune therapies can help repair or normalize bone tissue, and this will be discussed in the following sections.

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Bone targeted Agents: Radium, Desonumab and Zoledronic Acid

Targeting the bone microenvironment is now an established treatment for CRPC and three bone-directed agents have been incorporated into the clinical space, Radium-223, Desonumab, and Zoledronic acid. Radium-223 is a novel α-emitting radiopharmaceutical that demonstrated and overall survival benefit compared to placebo in a phase III trial of bone metastatic CRPC patients [58]. Desonumab is a human monoclonal antibody against RANKL, a key regulator of osteoclast function and survival. In a phase III trial, desonumab was superior to zoledronic acid in patients with mCRPC for preventing skeletal-related events by 3 months [59]. Whereas both groups were similar in overall survival impact. Zoledronic acid is a bisphosphonate that inhibits osteoclast-mediated bone resorption and accumulates in bone from binding to mineralized surfaces. Zoledronic acid has shown protective effects against skeletal related events, but no overall survival benefit [23,52]. Thus, these trials suggest that combination of bone-directed agents with immune therapies might be a potential strategy for improving clinical benefit for mCRPC patients. And although bone-directed agents have multiple effects on cells in prostate cancer bone metastases, they still lack the multifunctional effects necessary to impact both bone and tumor cells involved in the malignant interaction at the site of bone metastases (Figure 7). Another limitation of the current bone-directed agents is that they are not known to stimulate a robust CTL response on their own. Thus, an immune therapy that can augment effects of the current mCRPC therapeutics in bone and tumor will be a significant improvement in how mCRPC is treated. An intriguing new modality of immune therapy is proposed in the following section.

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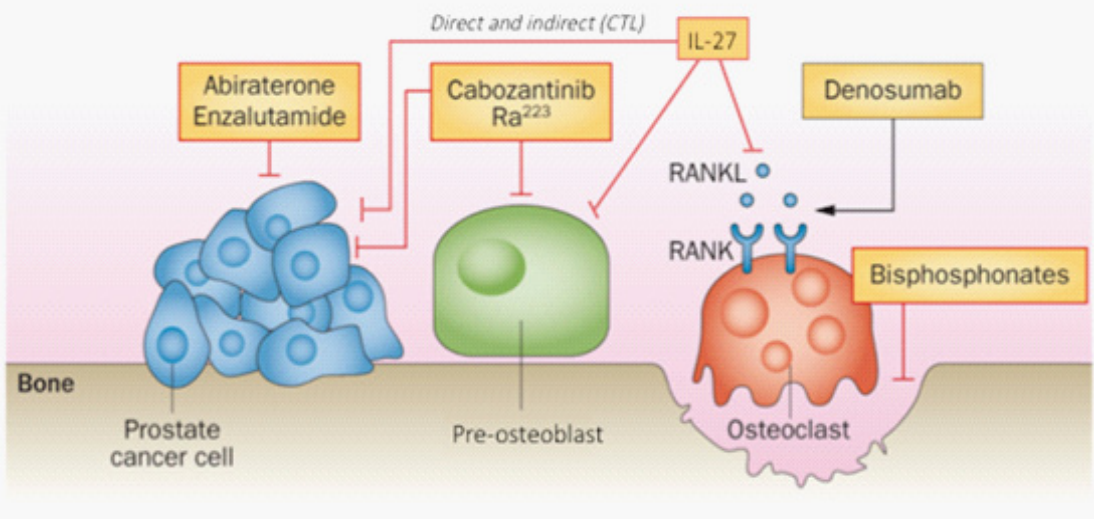


Figure 7: Therapeutics with promise for adaptation into the clinical space for stabilizing skeletal related events or promoting combined immunotherapy and bone lesion repair **(IL-27)**. The optimal therapeutic niche needs to be identified for immunotherapy in combination with current immunomodulatory chemotherapies. We anticipate that osteo-immunetherapies will begin to show a significant impact in the treatment of prostate and other bone-trophic cancer, since this modality may be efficient in reducing tumor burden while restoring bone, which will be an exciting prospect for cancer therapy. It will be important to examine the effectiveness of combination therapies in preclinical studies to examine whether osteo-immune cytokines like IL-27, which can positively impact both tumor therapy and bone repair, can prevent docetaxel relapse and/or whether low-dose chemotherapy that is immunomodulatory can augment immunotherapy efficacy. *Adapted by permission from Macmillan Publishers Ltd: Nat Rev Clin Oncol [60], copyright (2014).*

IL-27 Promotes Bone Normalization in Conjunction with an Anti-Tumorresponse.

An example of an immune therapystill at the preclinical stage but with promise for mCPRC include Interleukin-27 (IL-27), a multifunctional cytokine that drives antitumor activity through direct and indirect mechanisms (Figure 7). IL-27 drives immune-activation [61-64] and anti-angiogenic [65] mechanisms. Immune-activation occurs through stimulation of a STAT1/T-bet/IFNγ pathway [66], resulting in significant effector T cell recruitment in tumors (CD8, γδT, and NKT) as well as reduction of immune suppressive T and myeloid cells [9,64]. Moreover, IL-27 is a pro-osteogenic factor [9,67], as it inhibits osteoclast formation and increases the pool of mature functional osteoblasts, also through STAT1/IFNγ activation [9,67].

Novel methods have been developed for expression of IL-27 *in vivo* [9,64,68]. These methods have included ultrasound-assisted sonoporation delivery (sonodelivery) or electroporation of

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plasmid DNA into tumors or into skeletal muscle. The muscle delivery appears to be well-suited for treating metastatic tumors, since skeletal muscle can express and secrete high therapeutic levels of cytokines, which can reach often-inaccessible tumor bone metastases. The delivery and targeting of multifunctional cytokine IL-27 to the tumor/bone microenvironment has potential for disrupting key malignant crosstalk while inducing immune-activation to reduce tumor burden, restore bone, and increase survival.IL-27 might benefit from combination with agents that can attenuate TGFβ-mediated immune suppression in the bone metastatic microenvironment, providing critical adaptive surveillance and memory for eliminating relapsed tumor cells [69]. IL-27 and similar monotherapies may fare best in combination with endocrine disruptors [70], and particularly in a sequential administration, for example either following IMO induced by an endocrine disruptor (abiraterone), a second-generation taxane (cabazitaxel) or an emerging cytoreductive drug (cabozantinib). We anticipate that the current chemotherapeutic modalities for mCRPC will benefit greatly from combination with emerging treatments based on gene transfer technology such as IL-24 or IL-27. Interesting new modalities also include modified cytokines such as AM0010, a polyethylene glycol-modified (PEGylated) form of IL-10, currently under examination in a phase I trial (NCT02009449) [39]. It will be interesting to determine whether gene delivery or PEGylation of cytokines might be the optimal delivery strategy in the clinical space.

CONCLUSIONS

Immune therapy has generated excitement in the field of mCRPC treatment and has high potential in being effective in the challenging clinical space of current chemotherapeutics. Further improvement of the current approaches is required and may be achieved by combining T-cell- and antibody-based vaccination strategies with radio-, hormone-, chemo-, or antiangiogenic therapy. The field of immune therapy is evolving very quickly and the advance of genetic engineering has made several cancer vaccine treatments possible and also the delivery of cytokines via viral and non-viral vectors. Current trials are largely exploring immune therapy in combination with current chemotherapeutics. For example, Sipuleucel T is being examined in combination with androgen deprivation agents Abiraterone or Enzalutamide and also with immune checkpoint inhibitor IPI [42], and Radium-223 or radiation therapy, for example [39]. IPI is being evaluated with androgen suppression therapy or Abiraterone/prednisone, while Prostvac is being examined in conjunction with enzalutamide, docetaxel, or interestingly, radioisotope Samarium (^{153}Sm) lexidronam for alleviating bone pain associated with tumor bulk [39]. These various clinical trials are close to completion and some will have results within the next 1-2 years.

Also an emerging field, personalized oncology promises to innovate cancer therapy and target the previously undruggable space with cancer vaccines developed against the patient's own tumor antigens, as well as those incorporating adoptive T cell therapies and immune checkpoint inhibitor or immunogenic modulators to drive a personalized immune response against tumors. Developments also in biomarker-driven immuno-oncology and how individuals

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respond to immunetherapy will enable the next generations of designer or individualized cancer therapeutics. Future directions also will likely include utilizing personalized therapies such as individualized tumor-specific neoantigens predicted from sequencing biopsy material, providing targets of new T cells, leading to better responses to checkpoint inhibitor therapy.

Interestingly, the rise of immune therapy is happening at a time coincidental with the Cancer Moon Shot 2020 project, which intends to design, initiate and complete randomized clinical trials at all stages of cancer in up to 20 tumor types in as many as 20,000 patients by the year 2020. While not all these clinical trials will lead to viable therapeutic agents, there is great hope that these advances will help relegate mCPRC to a manageable chronic disease without severe suffering and death.

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