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Enhancing Efficacy of Therapeutic Vaccinations by Combination with Other Modalities

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Abstract

Novel strategies are emerging from preclinical and clinical investigations for combining vaccines with conventional and experimental anticancer therapies. Several lines of research show that combining either radiation or certain chemotherapeutic agents with vaccine can alter the phenotype of tumor cells, rendering them more susceptible to T-cell-mediated killing. Furthermore, there is emerging data suggesting that an immune response elicited by vaccine may augment the antitumor effectiveness of subsequent therapies. This article reviews and discusses therapeutic cancer strategies that employ vaccines sequentially or in combination with conventional cytotoxic therapies such as local radiation, chemotherapy, and hormone therapy, or immunopotentiating therapies such as anti-CTLA-4 monoclonal antibodies. Preliminary results of clinical studies using these combination strategies have demonstrated a postvaccination antigen cascade, prolonged time to disease progression, and evidence of improved overall survival. Large randomized studies are currently underway to further investigate these findings.

Keywords

Immunotherapy; vaccine; clinical protocols; prostate cancer; prostate-specific antigen; cancer vaccine; combination therapy; hormone therapy; chemotherapy; radiation therapy; anti-CTLA-4

2. Introduction

Therapeutic cancer vaccines as monotherapy for patients with large tumor burden have demonstrated only minimal clinical efficacy to date. In many instances, patients being treated with vaccines have undergone several prior therapeutic regimens, thus compromising their immune systems. Furthermore, tumor cells produce immunoregulatory substances that are able to anergize T cells. Tumor architecture, vasculature, and interstitial pressure impede T-cell penetration into large tumor masses. In a large tumor mass, expression of major histocompatibility complex (MHC) class I molecules may be reduced, and tumor cells far outnumber the antigen-specific T cells generated by the host immune system [1-3]. Thus, vaccines alone would probably be most effective as adjuvant or neoadjuvant therapy, and/or in patients with low tumor burden.

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Conflicts of Interest
The authors declare no conflicts of interest.

However, vaccines in combination with other therapies may be effective for both early-and late-stage disease. Conventional cytotoxic therapies may help overcome many of the immunologic hurdles that impede the success of vaccine therapy. Moreover, certain conventional therapies may normalize tumor vasculature, decrease interstitial pressure, up-regulate MHC molecules, tumor-associated antigens (TAAs), or Fas on tumor cells, any of which could facilitate immune-mediated recognition and destruction of tumor cells. In this article, we review and discuss multiple strategies for combining vaccines with conventional cancer therapies such as local radiation, chemotherapy, and hormone therapy, and with anti-CTLA-4 monoclonal antibody.

3. Vaccine and Radiation

One mechanism by which a tumor may escape immune destruction is the selection of tumor cells that have lower or no expression of MHC molecules or TAA, thus rendering even the generation of a robust immune response to vaccine insufficient to generate a clinical response. Radiation therapy in combination with vaccines is a promising strategy for overcoming these difficulties. Local tumor irradiation is the standard of care for many cancer types because of its direct cytotoxic effect on tumor cells. Due to limits in toxicity, some tumor cells within a given tumor mass may receive a sublethal dose of radiation. This dose, however, may be capable of modulating numerous classes of genes, resulting in phenotypic alteration of the tumor cells [4-6]. Genes that have been shown to be up-regulated postirradiation in murine and/or human tumors include Fas, MHC class I, ICAM-1, and multiple TAAs. Up-regulation of any one of these genes can potentially render tumor cells more susceptible to T cell-mediated immune attack.

Recent preclinical studies investigated the phenotypic and biologic effects of the irradiation of tumor cells on antigen-specific CTL killing, both in vitro and in vivo. Irradiation resulted in a durable up-regulation of cell surface expression of Fas and ICAM-1 in a dose-dependent manner in murine adenocarcinoma cell lines [7]. However, in vivo it is possible that these immune-potentiating phenotypic changes would not prove beneficial if radiation were to kill all the tumor-specific infiltrating lymphocytes. To study this, the authors showed that radiation was able to improve the lytic sensitivity of tumor cells by TAA-specific CTL. A subsequent study [8] showed that irradiation-induced up-regulation of Fas on tumor cells could be combined with an active immunotherapy regimen. CEA-transgenic mice bearing MC38-CEA murine colon tumors were given priming vaccinations of recombinant vaccinia (rV) containing the transgenes for CEA and a triad of costimulatory molecules (B7.1, ICAM-1, LFA-3, designated TRICOM), followed by recombinant fowlpox (rF)-CEA-TRICOM booster vaccines. All vaccinations were given with rF-GM-CSF. Treatment began 8 days after tumor transplantation. Radiation was initiated with 8 Gy once tumors reached a median volume of 75 to 100 mm^3. Neither radiation alone at this dose and schedule nor vaccine therapy alone inhibited tumor growth in the murine model. But local radiation and vaccine therapy used in combination had significant antitumor effects. Sublethal doses of radiation up-regulated surface expression of Fas, ICAM-1, and MHC class I on tumor cells in a dose-dependent manner. In addition, destruction of the MC38-CEA tumors by this combination regimen was shown to be associated with a massive infiltration of tumor-specific $CD8^+$ T cells [8].

Further preclinical studies showed similar findings with human cell lines [9]. Twenty-three human carcinoma cell lines (12 colon, 7 lung, 4 prostate) were subjected to 10 Gy of radiation. Seventy-two hours following radiation, changes in expression of surface molecules such as Fas, ICAM-1, MUC-1, CEA, and MHC class I were examined. Results showed that 91% (21/23) of cell lines up-regulated one or more of these surface molecules, which are involved in T cell-mediated immune attack postirradiation [9]. Furthermore 5 of 5 irradiated CEA^+/$HLA\text{-}A2^+$ human colon cancer cell lines analyzed demonstrated significantly enhanced killing

by CEA-specific HLA-A2-restricted CTLs compared with nonirradiated cell lines [9]. Radiation has also been shown to up-regulate chemokines elaborated by tumor cells [10], which can lead to improved T-cell trafficking to the tumor site. It is also known that low doses of radiation can induce stress genes and increase reactive oxygen species.

A recent clinical study reported on the use of a recombinant cancer vaccine combined with standard definitive radiotherapy in patients with localized prostate cancer (Table 1) [11]. The purpose of this trial was to determine if vaccine could induce an immune response in the presence of tumor irradiation. Because radiation alone can generate an inflammatory reaction, the trial was designed as a randomized phase II study, with patients receiving local definitive radiation with or without vaccine [11]. The primary endpoint of the trial was immunologic response, with secondary endpoints of safety and clinical response. Nineteen patients received vaccine plus radiation and 11 patients were given radiation alone. Patients in the combination arm received a priming vaccine of rV expressing prostate-specific antigen (rV-PSA) admixed with rV expressing the costimulatory molecule B7-1 (rV-B7-1), followed by monthly booster vaccines with rFPSA. The vaccines were given with local GM-CSF and low-dose systemic IL-2. Patients received standard external-beam radiation therapy between the fourth and sixth vaccinations. This regimen was well tolerated, with no grade 3 toxicities attributed to vaccine. However, many patients did develop transient toxicities to IL-2, resulting in dose reductions for the majority of patients. Of 17 patients in the combination arm who completed all 8 vaccinations, 13 had increases of at least 3-fold in circulating PSA-specific T cells. No detectable increases in PSA-specific T cells were seen in the radiotherapy-only arm ($p < 0.0005$). Patients in the combination arm also showed evidence of de novo generation of T cells to prostate-associated antigens not present in the vaccine (antigen cascade), providing indirect evidence of immune-mediated tumor killing. This clinical trial was the first to combine a vaccine with definitive external-beam radiation therapy for prostate cancer, and the first published clinical trial to study the effect of radiation therapy on specific immune responses.

The combination of radiation and vaccine is now being studied in patients with androgen-independent prostate cancer (AIPC) metastatic to bone. In a randomized phase II study at the National Cancer Institute (NCI), patients are receiving samarium-153 lexidronam (Quadramet®; Cytogen, Princeton, NJ), a bone-seeking radionuclide, alone or in combination with vaccines containing PSA-TRICOM [12,13]. Preclinical studies have shown that the FDA-approved dose of Quadramet® delivers the amount of radiation to bone required to induce immunopotentiating phenotypic changes in tumor cells. The NCI study is designed to determine if radionuclide plus vaccine can delay time to progression over radionuclide alone.

4. Vaccine and Chemotherapy

Cytotoxic chemotherapy agents play an important role in the conventional treatment of a broad array of solid tumors. Unfortunately, patients with most metastatic solid tumors are rarely cured with chemotherapy alone. These drugs have traditionally been administered at doses that produce myelosuppression, with episodes of both neutropenia and lymphopenia as the drugs are given in cycles. Contrary to traditional beliefs, it has recently been shown that vaccine therapy may be not only compatible, but synergistic with certain chemotherapies if used in appropriate scheduling regimens (Table 2). A number of chemotherapy drugs, as well as interferon, can up-regulate MHC class I and numerous TAAs on the surface of tumor cells [14-21]. In addition, antigen presentation to $CD8^+$ CTL usually involves proteolytic cleavage of antigen in the cytosol and delivery of epitope peptides onto MHC class I molecules in the endoplasmic reticulum via the heterodimeric peptide transporter TAP1/TAP2. In a recent study, Wang et al. demonstrated that IFN-alpha2b significantly modulates the balance of STAT1/STAT3 in tumor cells and host lymphocytes, leading to up-regulation of TAP2 and augmented host antitumor response [22]. Chemotherapy agents commonly used in cancer

therapy, such as 5-fluorouracil, have also been shown to up-regulate CEA and MHC class I in tumor cells. In an experimental melanoma model, systemic cyclophosphamide combined with local intratumoral injection of dendritic cells led to complete tumor regression [23]. In preclinical murine studies, the chemotherapy agents cyclophosphamide, doxorubicin, paclitaxel, and docetaxel [24] enhanced antitumor immune response to a whole tumor-cell vaccine [25]. The immune enhancement may occur through several possible mechanisms. Preclinical studies have suggested that doxorubicin increases macrophage antitumor activity [26], and doubling of splenic macrophages has been demonstrated 5 days after administration of doxorubicin [27,28]. In addition, docetaxel treatment has been shown to increase cytokine production, which promotes inflammation [29].

In addition to these proinflammatory changes, chemotherapy may inhibit negative regulatory cells. Increased levels of $CD4^+/CD25^{high}$ immune regulatory T cells have been found in cancer patients with large tumor burden. It is possible that depleting these regulatory T cells through systemic chemotherapy could improve the efficacy of cancer vaccines. Cyclophosphamide has also been shown to reduce both the level and function of regulatory T cells in preclinical and clinical studies [30-32]. Finally, certain chemotherapeutic agents may kill tumor cells in a way that promotes uptake by dendritic cells and subsequent activation of cytotoxic $CD8^+$ T cells [33]. In one study, it was shown that doxorubicin caused caspase 3-mediated apoptosis of colon cancer cell lines, which induced a strong immune response. In contrast, mitomycin C-killed cells did not evoke an immune response [34].

A number of issues arise when considering the combination of chemotherapy with vaccines. It may be advantageous to combine vaccine with chemotherapy earlier in the disease process, since the size of the tumor as well as the number of prior chemotherapies may impair the immune system. Furthermore, not all chemotherapeutic agents are compatible with vaccine. And finally, when combining vaccine with chemotherapy, dose scheduling can be critical [25,35,36]. More studies are needed to optimize the combined use of vaccine and chemotherapy.

One phase II clinical study of patients with metastatic AIPC randomized to vaccine alone or vaccine with low-dose docetaxel was recently reported [37]. The vaccine regimen consisted of a priming vaccine with rV-PSA admixed with rV-B7-1, followed by monthly booster vaccines with rF-PSA. The vaccines were given with local GM-CSF. The primary endpoint was to evaluate the relative change in PSA-specific CD8 T-cell precursors from baseline to day 85; i.e., to see if concurrent docetaxel (with dexamethasone) had any effect on generating an immune response to the vaccine. The median increase in T-cell precursors to PSA was 3.33-fold in both arms following 3 months of therapy. Immune responses to other prostate cancer-associated tumor antigens were also detected. Eleven patients who progressed on vaccine alone were allowed to cross over to receive docetaxel at time of progression. Median progression-free survival on docetaxel was 6.1 months after receiving vaccine, compared with 3.7 months for patients on the same regimen of docetaxel in a historical control at the same institution [38]. This was the first clinical trial to demonstrate that docetaxel can be safely combined with vaccine without inhibiting vaccine-specific T-cell responses. Furthermore, the results of this trial provided the hypothesis-generating finding that prior vaccination may lengthen patient response to docetaxel compared to docetaxel alone.

5. Can Vaccine Improve Subsequent Therapy?

The most promising aspect of vaccine therapy may be its ability to initiate a dynamic process of host immune response that may be exploited in subsequent therapies. Several clinical studies have provided evidence of this phenomenon. In a phase I study at the Dana-Farber Cancer Institute [39], 17 patients with advanced-stage progressive cancer received a plasmid/

microparticle vaccine directed against cytochrome P4501B1, which is overexpressed on most tumors. Ten of 11 patients who failed to develop immunity to the vaccine progressed on subsequent therapies. In contrast, 5 patients who did develop immunity to vaccine unexpectedly showed marked responses to salvage therapy administered on progression. In most cases, salvage therapy lasted at least a year. Other studies have shown that clinical response to chemotherapy may be enhanced by induction or augmentation of immune response to vaccine. In a study at the H. Lee Moffitt Cancer Center and Research Institute [40], 29 patients with extensive small cell lung cancer received an adeno-p53 vaccine. Most patients had disease progression, but 57.1% showed p53-specific T-cell responses postvaccination, and 61.9% had objective clinical responses to chemotherapy administered immediately after vaccine therapy.

This phenomenon was also exemplified in a randomized placebo-controlled phase III trial utilizing the sipuleucel-T vaccine. Sipuleucel-T consists of autologous antigen-presenting cells (APC) and a fusion protein composed of prostatic acid phosphatase and GM-CSF. This study involved 127 patients with metastatic asymptomatic AIPC using sipuleucel-T [41]. While progression-free survival (the primary endpoint) did not reach statistical significance ($p = 0.52$), overall survival was statistically significant ($p = 0.01$) between vaccine (25.9 months) and placebo (21.4 months). Some patients in this study and another nearly identical study went on to receive docetaxel at progression. There was a striking and statistically significant increase in overall survival with docetaxel treatment in patients having had prior vaccine ($n = 51$) vs. placebo ($n = 31$) [42]. The median survival was 34.5 months for patients who received vaccine followed by docetaxel. In contrast, the median survival was 25.4 months for patients who received placebo and subsequent treatment with docetaxel, a 9.1-month difference ($p = 0.023$, HR 1.9). These groups appeared to be well balanced based on their baseline prognostic factors, using an independently validated predictive nomogram [43].

It is tempting to speculate that chemotherapy augments immune responses through a variety of mechanisms. These could include destruction or decreased function of regulatory elements within the immune system (e.g. regulatory T cells), apoptosis of tumor cells in a way that stimulates the immune system, a decrease in immune regulatory substances elaborated by tumor cells, and alteration of the phenotypic characteristics of tumor cells, making them more amenable to immune-mediated recognition and destruction.

6. Vaccine and Androgen-Deprivation Therapy

There is growing interest in combining androgen-deprivation therapy (ADT) and vaccine in the treatment of prostate cancer. Mercader et al. showed that ADT induces profuse T-cell infiltration of benign glands and tumors in the human prostate [44]. T-cell infiltration was readily apparent after 1 to 3 weeks of therapy, and T cells within the treated prostate exhibited restricted T-cell receptor usage consistent with a local oligoclonal response. Studies have also shown that ADT is associated with enlargement of the thymus, enhancement of the T-cell repertoire, and abrogation of immune tolerance to the prostate [45-47]. Another study showed that an androgen receptor antagonist (ARA), flutamide, prevented testosterone-mediated inhibition of T-cell proliferation [48]. These studies have important implications for the use of vaccine in combination with hormone therapy in prostate cancer and other hormone-sensitive malignancies, including breast cancer.

There is no existing standard of care for patients with nonmetastatic AIPC. A phase II clinical trial in this patient population employed rV-PSA plus rV-B7-1 followed by rFPSA boosting [49]. Patients who were not surgically castrate remained on ADT and were randomized to vaccine ($n = 21$) versus ARA therapy with nilutamide ($n = 21$). After 6 months, patients with rising PSA and no metastasis could receive a combination of both treatments. The median pretreatment PSA velocity of 6.6 ng/mL/month decreased following 6 months of vaccine

therapy to 4.5 ng/mL/month (p = 0.025). Median time to treatment failure was 9.9 months in the vaccine arm versus 7.6 months in the nilutamide arm (p = 0.28). Twelve patients on the vaccine arm had nilutamide added at the time of PSA progression. Median time to treatment failure with the combined therapy was 13.9 months, for a total of 25.9 months from initiation of therapy. In contrast, 8 patients from the nilutamide arm had vaccine added at the time of PSA progression. Median time on study with the combined therapy was 5.2 months, with a total duration from onset of study of 15.9 months. Both vaccine and nilutamide appeared to have clinical activity, but patients appeared to respond better to nilutamide after receiving vaccine This study was the first to provide preliminary evidence that the combination of vaccine and hormone therapy may have more clinical benefit than either modality alone [49]. A follow-up analysis of this study showed that the 5-year survival rate was 75% for patients who received vaccine first then had nilutamide added, compared to a 43% 5-year survival rate for patients who started on nilutamide then had vaccine added [50].

As a result of the above study, a randomized phase II clinical trial has recently been initiated at the NCI combining the PSA-TRICOM vaccine with a second-line ARA (flutamide) in D0.5 prostate cancer patients compared to ARA alone [51]. This study was designed to determine if a combination of vaccine plus ARA prolongs time to treatment failure (defined as rising PSA, development of metastatic disease, or removal from treatment due to excessive toxicity) compared to ARA alone.

Preliminary results of a study combining the vaccine sipuleucel-T with ADT were recently announced, providing initial evidence of improved outcomes in patients with AIPC [41]. Sipuleucel-T, an autologous cell product pulsed with a GM-CSF-PAP fusion protein, was tested in a randomized, placebo-controlled trial in men with nonmetastatic hormone-sensitive prostate cancer who had biochemical recurrence following radical prostatectomy [52]. A total of 176 patients were randomized 2:1 to receive sipuleucel-T or placebo following a 3-month course of hormonal therapy. Patients were then followed with serial PSA measurements to evaluate the impact of sipuleucel-T on PSA and PSA doubling time (PSADT). Preliminary analysis showed a 35% increase in PSADT for patients in the sipuleucel-T arm compared to patients in the placebo arm (p = 0.046). To adjust for potential variations in the rate of testosterone recovery following hormone therapy, PSADT was calculated after testerone levels returned to baseline. These data showed that patients in the sipuleucel-T arm had a 49% increase in PSADT compared to patients in the placebo arm (p = 0.038).

7. Vaccine and Anti-CLTA-4 Monoclonal Antibody (mAb)

TAAs are weak antigens and therefore a signal from the T-cell receptor alone is insufficient for optimal T-cell activation [53]. A second signal from the APC is required for T-cell activation against a specific target. This signal is mediated by B7 on the APC and CD28 on the T cell. Once the T cell has been activated, CTLA-4, which is a T cell surface glycoprotein and CD28 homolog, is expressed and binds to B7. This higher-affinity binding of CTLA-4 for B7 generates a negative signal cascade and leads to down-regulation of the T-cell response. This combination of events is believed to moderate T-cell-mediated immune responses and tolerance to self-antigens [54-56].

Anti-CTLA-4 mAb was developed to bind to the CTLA-4 on T cells and therefore prevent the inhibitory cascade triggered by CTLA-4 binding to B7 [54,57,58]. In a murine model, anti-CTLA-4 mAb has demonstrated antitumor activity in moderately antigenic and highly immunogenic tumors [54]. Anti-CTLA-4 mAb alone is not sufficient to alter the growth of poorly immunogenic tumors such as MC38 [59,60]. Preclinical investigations have been performed using anti-CTLA-4 mAb in combination with cancer vaccines to increase the magnitude of the T-cell response and the avidity of antigen-specific T cells [58]. Initial studies

defined optimal doses of anti-CTLA-4 mAb when used in combination with rV-CEA-TRICOM to augment antigen-specific T-cell responses. Mice treated with rV-CEA-TRICOM and anti-CTLA-4 generated twice as many CEA-specific T cells as compared to vaccine alone, but the functional avidity (as measured by lytic ability) of the T cells increased by 10-fold [58]. The murine model also demonstrated that the combination of rV-CEA-TRICOM, anti-CTLA-4 mAb, and rFGM-CSF can work synergistically to decrease the size of CEA-expressing tumors [58]. A large phase I clinical trial using an anti-CTLA-4 mAb (ipilimumab; Medarex, Princeton, NJ) alone in 198 patients with metastatic melanoma or renal cell cancer was recently reported [61]. In addition to a 14% response rate, numerous immune-mediated side effects were seen, including nephritis, hepatitis, uveitis, panhypophysitis, dermatitis, and enterocolitis. Clinical trials using ipilimumab combined with a peptide vaccine showed antitumor activity in patients with melanoma, though severe but reversible immune breakthrough events (IBE) were seen [62].

Promising preliminary results of ipilimumab in combination with a whole tumor-cell vaccine have been seen in patients with asymptomatic AIPC [63]. Chemotherapy naïve patients were treated with GVAX® (Cell Genesys, South San Francisco, CA) every 2 weeks and ipilimumab in dose-escalating cohorts of 3 patients each every 4 weeks for up to 24 weeks. The combination therapy resulted in a greater proportion of patients with declines in PSA than either treatment alone. Five of 6 patients treated at the higher dose levels (3 mg/kg and 5 mg/kg) had decreases in PSA >50%. One patient had a PSA reduction from 50 ng/mL prior to treatment to 0.5 ng/mL after 2 months, along with resolution of retroperitoneal lymphadenopathy. Another patient with a >50% PSA decline also had substantial improvement on bone scan. Each patient who responded also had an accompanying IBE; several patients experienced a rise in PSA prior to the IBE and subsequent response. This and other emerging data reinforce the notion that the clinical benefit of immune-based therapies cannot be judged solely by conventional paradigms such as RECIST or strict rises in tumor markers such as PSA [64,65]. Studies such as these demonstrate that, in many cases, clinical responses may be seen only after several months of immunotherapy. Furthermore, patients with rapidly progressing disease, or those who have undergone multiple chemotherapy regimens and may thus have less functional immune systems, may not be the ideal population to evaluate the effectiveness of immune-mediated therapies. New assays and markers that could improve prospective selection of patients prior to immune therapy would be of great benefit in targeting new treatments to the appropriate patients.

8. Conclusion

Initial disappointing studies with vaccines alone in patients who had progressive disease following multiple cycles of chemotherapy have led to the investigation of combination strategies involving vaccines and conventional therapies. Results of initial clinical trials indicate that established cancer therapies such as chemotherapy and radiation may actually augment the potential for immune-mediated tumor destruction and clinically meaningful improvements. Unlike passive drugs that are metabolized and then eliminated, active immunotherapy strategies induce a dynamic response that can persist long after vaccination. Subsequent therapies may indeed augment this immune response without the need for further vaccinations. Future clinical trials designed to optimize combination therapies will be needed to definitively determine clinical benefit.

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Table 1

Results of clinical trials utilizing therapeutic vaccinations in combination with conventional therapies.

Patient Population	N	Treatment Regimen	Results	Ref
Localized prostate cancer	30	External beam radiation alone or in combination with poxviral-based PSA vaccine	17 pts completed therapy, 13 had ≥3-fold increase in T cells specific for PSA. Additional responses to other TAAs seen. These results were not seen in the 11 pts treated with radiation alone.	[11]
Metastatic AIPC	28	Poxviral-based PSA vaccine alone or in combination with docetaxel (with dexamethasone)	Both arms had a median 3.33-fold increase in PSA-specific T cells after 3 months of treatment. These results indicate the immune response was not mitigated by chemotherapy. Responses to other TAAs were seen. Patients treated with vaccine in combination with docetaxel had a longer OS than expected based on historical controls.	[37]
Advanced-stage cancer	17	Plasmid vaccine against cytochrome P4501B1 in combination with salvage regimen	5/6 pts who generated an immune response also had a sustained response to the salvage regimen. 2/5 responders sustained a complete response at 17- and 20-month follow-up, respectively.	[39]
Small cell lung cancer	29	Dendritic cells transduced with p53 vaccine via adenovirus	61.9% of pts treated with chemotherapy after vaccine had an objective response. 57.1% of pts generated a p53-specific T-cell response. Clinical response and OS were higher in pts who developed an immune response.	[40]
Nonmetastatic AIPC	42	Nilutamide vs. poxviral-based PSA vaccine, with cross-over to both at biochemical progression	Pts (n=12) who started with vaccine and crossed over to nilutamide at progression had a TTF of 25.9 months and 5-year OS of 75%. Pts (n=8) who started on nilutamide and crossed over to vaccine at progression had a TTF of 15.9 months and 5-year OS of 43%.	[49]
Metastatic AIPC	10	Whole tumor-cell vaccine in combination with anti-CTLA-4 antibody	5/6 pts treated with the higher dose levels had >50% decline in PSA. Some pts with PSA declines had decreases in bone and soft tissue lesions.	[63]
Symptomatic metastatic AIPC	600	Whole tumor-cell vaccine with docetaxel chemotherapy vs. chemotherapy alone	Trial accruing.	[66]

AIPC = androgen-independent prostate cancer; pts = patients; TAA = tumor-associated antigen; OS = overall survival; TTF = time to treatment failure

Table 2

Potential mechanisms of chemotherapeutic enhancement of immunotherapy.

Chemotherapeutic agent	Mechanism of enhancement
Fluorouracil	Changes tumor phenotype [23].
Cyclophosphamide	Decreases negative immunoregulatory cells [30].
Doxorubicin	Promotes caspase 3-dependent apoptosis [34].
Docetaxel	Increase in proinflammatory cytokines [67].