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Combining Vaccines with Conventional Therapies for Cancer

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Abstract

Preclinical and clinical investigations currently underway are employing novel strategies for combining vaccines with conventional and experimental anticancer therapies. To date, the FDA has not approved a therapeutic cancer vaccine. However, the results of recent investigations suggest an increasing role for vaccines in new models of combination therapy for many types of cancer. This article reviews and discusses therapeutic cancer strategies that employ vaccines in combination with local radiation, chemotherapy, hormone therapy, and anti-CTLA-4 mAb. Preclinical studies have shown that certain anticancer agents have immune modulatory effects that result in up-regulation of surface expression of MHC molecules, tumor-associated antigens, or Fas on malignant cells, rendering them more susceptible to immune destruction. Preliminary results of clinical studies using combination strategies have demonstrated a postvaccination antigen cascade, prolonged time to disease progression, and improved overall survival. Several larger randomized trials are ongoing, and more are required to support these findings.

Keywords

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

Introduction

Recent preclinical and clinical studies have supported the rationale for combining vaccines with conventional therapies for patients with metastatic cancer. Anticancer vaccines as monotherapy for patients with large tumor burden have demonstrated only minimal clinical efficacy. In many instances, patients being treated with vaccines have undergone several prior therapeutic regimens, thus compromising their immune systems. Furthermore, tumor cells produce immunoregulatory molecules that are able to anergize T cells. Tumor architecture, vasculature, and interstitial pressure make it difficult for T cells to penetrate 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.

Preclinical studies lend support to the rationale of combining vaccines with conventional therapies. In vitro studies have shown that certain anticancer agents have immune modulatory effects that result in up-regulation of cell surface expression of MHC molecules, tumor-associated antigens (TAAs), or Fas on malignant cells, rendering them more susceptible to immune destruction. We review and discuss here multiple strategies for combining vaccines with conventional cancer therapeutic modalities, such as local radiation, chemotherapy, and hormone therapy, and with anti-CTLA-4 mAb (Table 1).

Vaccine Plus Radiation

Local radiation is the standard of care for many cancer types because of its direct cytotoxic effect on tumor cells. It is usually employed as a therapeutic and/or palliative strategy, but may also be used to alter tumor architecture, which results in more effective drug delivery. Due to limits in toxicity, some tumor cells within a given tumor mass often receive a sublethal dose of radiation; this dose, however, may modulate 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 the TAAs CEA, MUC-1, HER-2/neu, p53, and CA125. Up-regulation of any one of these genes can potentially render tumor cells more susceptible to T cell-mediated immune attack. For example, when Fas, a member of the tumor necrosis factor receptor family, binds to its natural ligand (FasL), it induces apoptosis. Fas-mediated apoptosis, along with granzyme-mediated killing, has been shown to play an important role in the immune destruction of tumor cells.

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. The model used was a murine adenocarcinoma cell line (MC38) that expressed CEA (MC38-CEA). This cell line expresses low levels of Fas and is weakly sensitive to antigen-specific CTLs. Initial studies demonstrated that irradiation up-regulated cell surface expression of Fas and ICAM-1 in both the CEA^- parental cell line MC38 and in MC38-CEA cells in a dose-dependent manner.[7] The up-regulation of Fas was durable (>96 hours) and daughter cells continued to express higher levels of Fas for at least 4 generations. In this study, radiation's ability to improve the lytic sensitivity of tumor cells was defined employing a CEA-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 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. One 8-Gy dose of local radiation induced up-regulation of Fas in situ for up to 11 days. Clinically, irradiation of tumors is routinely fractionated into daily doses because of potential damage to normal tissues. When MC38-CEA tumors were subjected to 8 Gy of radiation delivered as 2 Gy/day for 4 days, the up-regulation of Fas was comparable to that of a single 8-Gy dose. Neither radiation at this dose 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. Destruction of MC38-CEA tumor cells by this combination regimen was shown to be associated with a massive infiltration of $CD8^+$ T cells.[8]

These preclinical studies were extended to determine if sublethal doses of radiation would alter the phenotype of human tumor cells and render them more susceptible to T cell-mediated attack.[9] Twenty-three human carcinoma cell lines (12 colon, 7 lung, 4 prostate) were subjected to sublethal (10 Gy) doses of radiation. Seventy-two hours postirradiation, changes in

expression of surface molecules involved in T cell-mediated immune attack, 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 postirradiation.[9] While no consistent pattern of up-regulation was observed across the group, 5/5 irradiated $CEA^+/A2^+$ human colon tumor cell lines analyzed demonstrated significantly enhanced killing by CEA-specific HLA-A2-restricted CTLs compared with nonirradiated cell lines.[9] Significantly, one of the colon tumor cell lines examined, SW620, is defective in Fas signaling, and thus cross-linking of Fas does not result in increased cell death. Sublethal (10 Gy) radiation made this cell line significantly more susceptible to CTL killing, demonstrating that even in the absence of Fas, other phenotypic changes postirradiation can enhance tumor cells' susceptibility to attack. 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.[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 rF-PSA. 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 reported grade 3 toxicities 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 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, which was not powered to look definitively at clinical endpoints, 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. Patients in the vaccine arm had a median follow-up of 20 months; 2 of 17 evaluable patients had biochemical failure. Patients in the radiation-only arm had a median follow-up of 25.1 months. Two of 9 evaluable patients developed biochemical failure at 17 and 24 months after initiation of radiotherapy.

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. 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.

Vaccine Plus Chemotherapy

It has recently been shown that, although counterintuitive, vaccine therapy may be not only compatible, but synergistic with certain chemotherapies if used in appropriate scheduling regimens (Table 2). Drugs such as interferon can up-regulate both MHC class I and numerous TAAs on the surface of tumor cells.[12–19] It has also been shown that some drugs commonly used in cancer therapy can up-regulate tumor antigens and/or histocompatibility antigens. For example, 5-fluorouracil has 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.[20] In preclinical murine studies, the chemotherapy agents cyclophosphamide, doxorubicin, paclitaxel, and docetaxel[21] enhanced antitumor immune response to a whole tumor-cell vaccine.[22] In addition, 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 also improve the efficacy of cancer vaccines. 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.[23] Casares et al. showed 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.[24]

Using chemotherapy in combination with vaccine raises several important considerations. First, patients with advanced disease who have received multiple regimens of different chemotherapeutic agents undoubtedly have impaired immune systems as a result. Thus, it may be advantageous to combine vaccine with chemotherapy earlier in the disease process, when the immune system is still highly functional. Second, not all chemotherapeutic agents are compatible with vaccine. And finally, when combining vaccine with chemotherapy, dose scheduling can be critical.[22, 25, 26] Clearly, more studies are needed to optimize the combined use of vaccine and chemotherapy.

Arlen et al. recently reported a phase II clinical study of patients with metastatic AIPC randomized to vaccine alone or vaccine with low-dose docetaxel.[27] 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. Secondary endpoints included safety of the combination therapy, as well as clinical outcomes. 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.[28] 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 preliminary evidence that prior vaccination may lengthen patient response to docetaxel compared to docetaxel alone. As a consequence of these studies, a randomized phase II trial has recently been initiated at the NCI to compare the clinical benefit of docetaxel plus vaccine versus docetaxel alone in patients with metastatic breast cancer.[29]

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,[30] 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,[31] 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.

Vaccine Plus Hormone Therapy

There is growing interest in combining androgen-deprivation therapy (ADT) and vaccine in the treatment of prostate cancer. Kwon et al. showed that ADT induces profuse T-cell infiltration of benign glands and tumors in the human prostate.[32] 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. Other studies have shown that ADT is associated with enlargement of the thymus, enhancement of the T-cell repertoire, and abrogation of immune tolerance to the prostate.[33, 34] 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.[32]

There is no existing standard of care for patients with D0.5 prostate cancer (AIPC with rising PSA and no radiographic evidence of disease). A phase II clinical trial in patients with D0.5 prostate cancer employed rV-PSA plus rV-B7-1 followed by rF-PSA boosting.[35] Patients who were not surgically castrate remained on ADT and were randomized to vaccine (n = 21) versus androgen-receptor antagonist (ARA) therapy with nilutamide (n = 21). After 6 months, patients with rising PSA and no metastasis could receive a combination of both treatments. The primary endpoint of the study was to compare time to treatment failure for patients receiving vaccine versus patients on ARA. Secondary endpoints included immune response to vaccine, vaccine safety, and the effects of combining the 2 modalities in patients with progressive biochemical failure without metastasis. 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. The 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. The 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. To our knowledge, this was the first clinical trial devoted to patients with D0.5 prostate cancer and the first to provide preliminary evidence that the combination of vaccine and hormone therapy may have more clinical benefit than either modality alone.[35] A post-5-year 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.[36]

As a result of the above study, a clinical trial has recently been initiated at the NCI that combines PSA-TRICOM vaccines plus GM-CSF with second-line ARA (flutamide) in D0.5 prostate cancer patients. Patients will be randomized to receive either vaccine plus ARA or ARA alone. Flutamide will be discontinued at PSA progression and patients will either continue vaccine

or have vaccine initiated at that time. The primary endpoint of this randomized phase II clinical trial is 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. Secondary endpoints of the trial include determining toxicity, as well as PSA-specific T-cell responses in patients with PSA progression. The study will also evaluate patterns of immunologic effects that differ by treatment, including the immunologic effects of flutamide withdrawal. Results of this study may provide the rationale for a definitive phase III study employing this treatment strategy.

Vaccine Plus Anti-CLTA-4 mAb

For most weak antigens, such as TAAs, a signal from the T-cell receptor is insufficient for optimal T-cell activation.[37] A second immune-enhancing signal mediated from B7 on the antigen-presenting cell through CD28 on the T cell is required to activate the T cell specific for the target antigen. CTLA-4 is also expressed on the surface of the T cell 2 to 3 days following activation and also binds to B7. This higher-affinity binding of CTLA-4 for CD28 generates a negative signal, effectively diminishing the immune response. Anti-CTLA-4 mAb blocks the inhibitory action of CTLA-4 and also selects for higher-affinity T-cell clones.[38, 39, 40] Anti-CTLA-4 mAb has demonstrated antitumor effects in moderately antigenic and highly immunogenic murine tumors.[38] However, anti-CTLA-4 mAb alone does not significantly influence the growth of poorly immunogenic tumors such as MC38.[41, 42] Hodi et al., published a Phase I clinical study utilizing anti-CTLA-4 as a therapeutic modality. Nine advanced cancer patients previously treated with vaccine therapy for either melanoma (n=7) or ovarian carcinoma (n=2) were assessed for biologic activity and toxicity of this therapy.[43] A dose of 3mg/kg of MDX- CTLA-4 was administered. No serious toxicity occurred, although T-cell responses were observed against normal melanocytes. Three melanoma patients previously treated with an autologous GM-CSF secreting tumor cell vaccine demonstrated extensive tumor necrosis with immune infiltrates. This study suggested that prior immune memory responses may be amplified utilizing an anti-CTLA-4 antibody.[43]

Recent preclinical studies have explored the ability of anti-CTLA-4 mAb to alter the level and/or avidity of antigen-specific T cells when used in combination with vaccine.[40] Initial studies sought to define optimal dose scheduling of anti-CTLA-4 mAb with both rV-CEA-TRICOM and rF-CEA-TRICOM to enhance T-cell responses. Vaccinating mice with rV-CEA-TRICOM alone or rV-CEA-TRICOM plus anti-CTLA-4 mAb generated T cells with similar tetramer-positive precursor frequencies. Although there was a <2-fold increase in CEA-specific T cells in mice vaccinated with rV-CEA-TRICOM versus rV-CEA-TRICOM plus anti-CTLA-4 mAb, there was a profound difference in tetramer dissociation and a 10-fold increase in functional avidity in T cells receiving both rV-CEA-TRICOM and anti-CTLA-4 mAb.[40] In preclinical mouse tumor studies, the combined use of rV-CEA-TRICOM, anti-CTLA-4 mAb, and rF-GM-CSF resulted in synergistic reduction of CEA-expressing tumors.[40] In clinical trials involving patients with melanoma, anti-CTLA-4 mAb (ipilimumab; Medarex, Princeton, NJ) combined with a peptide vaccine showed antitumor activity accompanied by severe but reversible immune breakthrough events, including colitis and panhypophysitis.[43 44]

Preliminary results of a whole tumor-cell vaccine combined with ipilimumab were recently presented.[44 45] Patients with asymptomatic metastatic AIPC who were chemotherapy naïve were enrolled and treated with GVAX® (Cell Genesys, South San Francisco, CA) every 2 weeks and dose escalation of ipilimumab in cohorts of 3 patients each every 4 weeks for up to 24 weeks. Five of 6 patients comprising the highest 2 dose levels (3 mg/kg and 5 mg/kg) had decreases in PSA >50%. Each of the responding patients had an immune breakthrough event, including one patient whose prestudy PSA level was 50 ng/mL. After 2 months of treatment, this patient's PSA had decreased to 0.5 ng/mL, with resolution of retroperitoneal adenopathy.

Another patient whose PSA decreased >50% had a significant improvement in lesions on bone scan. The combination therapy yielded a greater proportion of patients with declines in PSA than either treatment modality alone. Interestingly, several of these patients experienced an initial worsening of disease, as measured by PSA, before mounting a subsequent dramatic response that correlated with the onset of an immune breakthrough event. In addition, emerging data reinforce the notions that clinical responses are more likely only after several months of therapy, and less likely in patients with rapidly advancing disease who have undergone chemotherapy and whose immune systems are thus comparatively less functional than patients who have had no prior chemotherapy. In an ongoing trial at the NCI (05-C-0167) involving ipilimumab and vaccine, patients who had received no prior chemotherapy were considerably more likely to have a clinical response and to stay on trial longer.

Conclusion

Research in molecular biology and immunology has resulted in the development of a range of recombinant vaccines, including viral-based vaccines, that encode TAA along with T-cell costimulatory molecules or cytokines for use in active immunotherapy. Evidence is emerging that vaccines will work synergistically with established cancer therapies such as chemotherapy, surgery, immunotherapy, and radiation. Thus, there is a need for relevant preclinical and early clinical studies to further evaluate these approaches. Future clinical trials will also need to incorporate more extensive monitoring of immune responses to help determine how vaccines induce effective tumor immunity, and to validate specific assays that correlate with clinical responses. Finally, almost all of the clinical trials of cancer vaccines have been in patients with advanced-stage disease. The ability of these vaccines to prolong survival in patients with early-stage disease and low tumor burden needs to be further explored.

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Table 1

Results of clinical trials combining vaccine with conventional therapies.

Disease	Treatment	Results (pts = patients)
Localized prostate cancer (n = 30)[11]	External beam radiation +/− poxviral-based PSA vaccine	13/17 pts who completed therapy had ≥ 3-fold increase in PSA-specific T cells. This result was not seen in the 11 pts treated with radiation alone. Responses to other tumor antigens were seen.
Metastatic androgen-independent prostate cancer (n = 28)[27]	Poxviral-based PSA vaccine +/− docetaxel (with dexamethasone)	Median 3.33-fold increase in PSA-specific T cells in both arms after 3 months of therapy, indicating chemotherapy did not mute immune response. Responses to other tumor antigens were seen. Median overall survival in vaccine + docetaxel arm was longer than expected based on historical controls.
Advanced-stage cancer (n = 17)[30]	Plasmid vaccine against cytochrome P4501B1 + salvage therapy	5/6 pts who generated immune response had sustained response to salvage therapy. 2 of those 5 maintained complete response at 17- and 20-month follow-up.
Small cell lung cancer(n = 29)[31]	Dendritic cells transduced with p53 vaccine via adenovirus	57.1% of pts had p53-specific T-cell response. 61.9% of pts treated with chemotherapy after vaccine therapy had objective response. Clinical response and survival were higher in pts who developed immune responses.
D0.5 prostate cancer (n = 42)[35]	Poxviral-based PSA vaccine vs. nilutamide, with cross-over to both at biochemical progression	Pts who started with vaccine and crossed over to nilutamide at progression (n = 12): time to treatment failure 25.9 months; 5-year overall survival rate 75%. Pts who started on nilutamide and crossed over to vaccine at progression (n = 8): time to treatment failure 15.9 months; 5-year overall survival rate 43%.
Metastatic androgen-independent prostate cancer (n = 10)[44]	Whole-cell vaccine + anti-CTLA-4 antibody	5/6 pts at higher dose levels had >50% decline in PSA. Bone and soft tissue disease also showed response to treatment.

Table 2

Potential mechanisms of chemotherapeutic enhancement of immunotherapy.

Chemotherapeutic agent	Mechanism of enhancement
Fluorouracil	Changes tumor phenotype.[20]
Cyclophosphamide	Decreases negative immunoregulatory cells.[46]
Doxorubicin	Promotes caspase 3-dependent apoptosis.[24]