Published in final edited form as:

Expert Opin Investig Drugs. 2010 February ; 19(2): 311–314. doi:10.1517/13543780903530678.

Combining low-dose cyclophosphamide with GM-CSF–secreting prostate cancer immunotherapy enhances antitumor immune effects

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Abstract

Prostate GVAX[®] is an allogeneic cell-based prostate cancer vaccine engineered to secrete GM-CSF. The release of GM-CSF by this immunotherapy serves to recruit dendritic cells, which then present tumor antigens to T cells, thus initiating antitumor immune responses. However, preclinical data show that, when used alone, cell-based immunotherapy is generally unable to break specific T-cell tolerance in tumor-bearing hosts. The study by Wada and colleagues employed an autochthonous prostate cancer mouse model to demonstrate that low-dose cyclophosphamide given prior to a cell-based GM-CSF–secreting vaccine (T-GVAX) abrogated immune tolerance, augmented prostatic CD8⁺ T-cell infiltration, mediated depletion of regulatory T cells (Tregs), and increased expression of dendritic cell maturation markers. In addition, this combination decreased the wet weight of mouse prostate glands, lowered histological tumor scores, and increased the density of apoptotic bodies. These findings add to existing data from other preclinical models showing enhancement of antitumor immunity when cyclophosphamide is administered in sequence with GM-CSF–secreting immunotherapy for the treatment of breast and pancreatic cancers. These studies provide a rationale for designing clinical trials that combine low-dose cyclophosphamide with GM-CSF–secreting cell-based immunotherapy in patients with prostate and other cancers.

Keywords

antitumor immunity; cyclophosphamide; GVAX[®]; immunotherapy; prostate cancer

1. Introduction

Cancer immunotherapy refers broadly to approaches that attempt to treat or cure cancer by activating an immune response directed against malignant tissue. A number of different

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Evaluation of: Wada S, Yoshimura K, Hipkiss EL, et al. Cyclophosphamide augments antitumor immunity: studies in an autochthonous prostate cancer model. *Cancer Res*. 2009;69:4309–18

Declaration of interest

The authors state no conflict of interest and have received no payment in preparation of this manuscript.

immunotherapy strategies have been developed for the treatment of prostate cancer including tumor cell vaccines, dendritic cell vaccines, genetic vaccines, and peptide vaccines. One approach involves the use of two irradiated allogeneic prostate cancer cell lines (LNCaP and PC3) that have been genetically modified to produce GM-CSF: a platform known as prostate GVAX[®] [1]. This strategy results in recruitment and activation of antigen-presenting cells (e.g., dendritic cells) to the vaccination site where uptake and processing of tumor antigens occurs. In lymphoid tissues, these activated dendritic cells present tumor-derived antigens to T cells, which then traffic to the sites of disease and (if successful) initiate an antitumor immune response. Countering these effects, cancers have evolved several mechanisms to escape immune surveillance, effectively inducing immune tolerance. For example, tumors have been shown to induce expansion of CD4⁺ CD25⁺ regulatory T cells (Tregs), leading to delayed or absent recognition of immunogenic cancers. Conversely, elimination of these Tregs often elicits potent antitumor lytic responses leading to tumor eradication.

For many years, the alkylating agent cyclophosphamide has been studied for its role in breaking immune tolerance when used at low (immunomodulatory) rather than high (cytotoxic) doses. However, the combination of low-dose cyclophosphamide with prostate GVAX[®] had not previously been investigated. To explore the immunomodulatory effects of cyclophosphamide when administered in combination with GM-CSF-secreting cancer immunotherapy, Wada and colleagues [2] used an autochthonous murine system based on transgenic adenocarcinoma of the mouse prostate (TRAMP) mice crossed with analogous transgenic mice that expressed the antigen hemagglutinin (HA) in a prostate-restricted manner (ProHA). This double transgenic murine system (designated ProHA × TRAMP) represents a clinically relevant animal model that spontaneously develops prostate tumors expressing HA as a unique tumor-associated antigen. The study [2] aimed to determine the optimal timing and dosing of immunomodulatory cyclophosphamide in relation to prostate GVAX[®], and to clarify the mechanism of immune activation using this combination.

2. Results

Through experiments involving adoptive transfer of prostate/prostate cancer-specific CD8⁺ cells as well as through studies of the endogenous T-cell repertoire, Wada and co-workers [2] showed that giving cyclophosphamide in sequence with cell-based GM-CSF-secreting immunotherapy (T-GVAX) resulted in potent augmentation of HA-specific CD8⁺ T-cell responses, both in the prostate gland and in the prostate-draining lymph nodes of ProHA × TRAMP mice. Experiments to determine optimal sequencing of the two agents demonstrated that maximal CD8⁺ T-cell expansion was observed when cyclophosphamide was given 1 day prior to T-GVAX, while administering cyclophosphamide on day -4, day -2, day +1, or day +2 in relation to T-GVAX was not as effective in inducing antitumor immunity.

Using this treatment sequence, several different dose levels of cyclophosphamide were explored with respect to antitumor immune responses: 12.5, 25, 50, 100, and 300 mg/kg (all given intraperitoneally). The dose of cyclophosphamide that resulted in the greatest HA-specific CD8⁺ T-cell response was found to be 50 mg/kg. Of note, this dose produced serum

levels roughly equivalent to those achieved in humans using a cyclophosphamide dose of 200 mg/m² (given intravenously). Importantly, higher doses of intraperitoneal cyclophosphamide in this animal model resulted in a loss of immunotherapy efficacy and a suppression of specific CD8⁺ T-cell expansion.

Next, different permutations of T-GVAX and cyclophosphamide were evaluated: these included T-GVAX monotherapy, cyclophosphamide monotherapy, cyclophosphamide sequenced with T-GVAX, and a negative control group receiving no therapy. In these experiments, it was shown that the combination of the two immune-activating agents produced markedly superior HA-specific CD8⁺ T-cell responses in both the prostate and the prostate-draining lymph nodes, than was seen when either modality was used alone (> 8-fold augmentation). This finding is particularly important, as it further demonstrates the weak antitumor immunogenicity of T-GVAX when used as monotherapy against prostate cancer.

Further mechanistic experiments showed that immune enhancement by low-dose cyclophosphamide was associated with a transient depletion of CD4⁺ FOXP3⁺ regulatory T cells in the prostate and prostate-draining lymph nodes of these mice. However, alterations in Treg function with the addition of cyclophosphamide were not observed. The combined treatment also resulted in increased ratios of CD4⁺ and CD8⁺ effector T cells to regulatory T cells (Teff/Treg ratio) in the prostate gland as well as in the spleen. In addition, the combination of T-GVAX and cyclophosphamide resulted in increased expression of dendritic cell maturation markers, although a dramatic change in dendritic cell numbers was not observed.

Finally, antitumor effects were investigated by examining the size of murine prostate glands, the histological appearance of prostate tumors, and the degree of tumor apoptosis. To this end, mice treated with the combination of cyclophosphamide and T-GVAX had smaller prostate gland wet weights (a surrogate measure of tumor burden), lower histological tumor scores (a surrogate measure of Gleason sum), and a greater number of apoptotic bodies compared with mice receiving monotherapy or no therapy.

3. Discussion and significance

The use of cyclophosphamide combined with vaccination has previously been investigated in other preclinical tumor models. One such example is the HER-2/neu transgenic mammary tumor mouse model that develops spontaneous HER-2/neu-expressing mammary tumors due to transgene activation by the murine mammary tumor virus (MMTV) promoter [3]. In this clinically relevant model of human breast cancer, it has been demonstrated that vaccination alone induces weak HER-2/neu-specific T-cell responses incapable of controlling mammary tumor growth, regardless of the vaccine type. However, treatment of these mice with low-dose cyclophosphamide prior to vaccination results in tumor rejection in 10 – 30% of animals, while all mice receiving vaccination alone become tolerant to tumor growth [3]. In this study, it was shown that the immune-enhancing effect of cyclophosphamide was mediated through depletion of CD4⁺ CD25⁺ Tregs and recruitment of antitumor effector T cells. These findings were confirmed in a separate study using the same HER-2/neu transgenic mouse model [4]. In that study, combined treatment with cyclophosphamide and

a GM-CSF-secreting whole-cell breast cancer vaccine delayed tumor growth by overcoming immune tolerance and inducing antigen-specific antitumor immunity [4]. Importantly, because cyclophosphamide exerts its effect on cells of the immune system (Tregs) rather than on cancer cells themselves, this approach can be applied to any type of cancer.

These studies have led to a Phase I/II clinical trial testing the immunogenicity of cyclophosphamide combined with a GM-CSF-secreting cell-based vaccine in patients with breast cancer [5]. In this study, 21 women with metastatic disease received one of three doses of cyclophosphamide (200, 250, or 350 mg/m²) in conjunction with doxorubicin and an allogeneic, HER-2/neu⁺, GM-CSF-secreting breast tumor vaccine. In addition, six women received immunotherapy alone. Patients on the combined-modality arms received three monthly immunizations according to the following treatment schedule: cyclophosphamide on day -1, vaccination on day 0, and doxorubicin on day +7. Total Her-2-specific immune responses were observed in 33, 50, 21, and 25% of patients receiving 0, 200, 250, and 350 mg/m² of cyclophosphamide, respectively [6]. These data suggest that addition of cyclophosphamide enhances vaccine responses in patients with breast cancer, but that increasing doses of cyclophosphamide may abrogate these immune effects.

A second study based on these principles has recently been conducted in patients with metastatic pancreatic cancer [7]. In this trial, 30 patients received up to six doses of a GM-CSF-secreting cell-based pancreatic cancer vaccine at 21-day intervals, while 20 patients received 250 mg/m² of intravenous cyclophosphamide 1 day prior to the same vaccine. After three cycles of therapy, pancreatic cancer-specific (mesothelin-specific) CD8⁺ T-cell responses were augmented in only 50% of patients treated with vaccine alone, compared with 90% of patients treated with cyclophosphamide plus vaccine. Mean immune response augmentation (as judged by mesothelin-specific CD8⁺ T-cell effects) was 39% in patients receiving immunotherapy alone and 131% in patients receiving cyclophosphamide plus immunotherapy [7]. In addition, median survival in patients on the vaccine arm was 2.3 months compared to 4.3 months for those on the cyclophosphamide/vaccine arm.

In summary, the study by Wada and colleagues [2], as well as the other preclinical and clinic studies summarized here, provide a strong rationale for the design of clinical trials using immunomodulatory cyclophosphamide in sequence with allogeneic cell-based GM-CSF-producing vaccines in several human cancers, including prostate cancer. These initial studies provide preliminary suggestions regarding the most effective sequencing of these two agents and the optimal dosing of cyclophosphamide, but require confirmation in early-phase human clinical trials.

4. Expert opinion and conclusion

Two large randomized Phase III studies of prostate GVAX[®] have been conducted. The first study, VITAL-1, enrolled 626 men with asymptomatic metastatic castration-resistant prostate cancer and randomized them to receive GVAX[®] or docetaxel/prednisone (the standard-of-care) [8]. The second study, VITAL-2, was designed to enroll 600 patients with symptomatic metastatic castration-resistant disease, randomizing them to docetaxel/

prednisone or docetaxel/GVAX[®] [9]. Both trials were terminated early: VITAL-1 because of data from an interim analysis suggesting that the overall survival improvements initially hypothesized were unlikely to be seen, and VITAL-2 because of an apparent higher mortality in the docetaxel/GVAX[®] arm [8,9]. While several potential reasons for this low activity may be postulated, one of the most compelling is the inability of this vaccine to overcome immune tolerance when used alone [10]. In addition, numerous clinical and preclinical studies suggest that cancer immunotherapy might prove more efficacious in a minimal-disease setting.

Building on the preclinical and clinical data summarized in this paper, investigators at our institution have designed a randomized neoadjuvant pilot study to assess the antitumor immune effects of prostate GVAX[®] monotherapy versus the combination of low-dose cyclophosphamide with GVAX[®], in patients with localized prostate cancer. These modalities will be administered 3 weeks prior to radical prostatectomy in men with intermediate- and high-risk disease, using pathological end points as the primary readout. Through this approach, we aim i) to determine whether prostate GVAX[®] used alone is immunogenic in men with localized prostate cancer by evaluating CD8⁺ T-cell infiltration (and other immunological and pathological end points) in harvested prostate glands; ii) to determine whether low-dose cyclophosphamide given intravenously 1 day prior to prostate GVAX[®] augments immune infiltration into the prostate; and iii) to investigate the safety and feasibility of this combinatorial immunologic strategy. We hypothesize that the sequencing of cyclophosphamide with GVAX[®] will produce significantly greater antitumor immune responses than single-agent GVAX[®], mirroring the findings in the ProHA × TRAMP model.

If this initial study shows significant enhancement of antitumor immunity when using low-dose cyclophosphamide in conjunction with GVAX[®] (and this combination is feasible and safe), then future studies will aim to use this approach in both the adjuvant and the salvage settings. For example, a clinical trial could be designed to assess the efficacy of cyclophosphamide/GVAX[®] given before and after radical prostatectomy in high-risk patients, using PSA relapse as the primary end point. Alternatively, a trial of cyclophosphamide/GVAX[®] combined with androgen ablation for patients with PSA-recurrent disease could be designed, with metastatic progression as the primary end point.

We are entering a new era of cancer immunotherapy. The prior notion of single-agent vaccination in patients with advanced disease is being replaced by the concept of combinatorial immunological strategies in patients with lower disease burden. Combining prostate GVAX[®] with low-dose cyclophosphamide in patients with non-metastatic prostate cancer may resurrect GVAX[®] immunotherapy from the grave, but confirmation of preclinical findings in the clinical arena is now imperative.

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