The Tipping Point for Combination Therapy: Cancer Vaccines with Radiation, Chemotherapy, or Targeted Small Molecule Inhibitors

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Abstract

Therapeutic cancer vaccines are a unique treatment modality in that they initiate a dynamic process of activating the host immune system, which can then be exploited by concurrent or subsequent therapies. The addition of immunotherapy to standard-of-care cancer therapies has shown evidence of efficacy in preclinical models and in the clinical setting. This review examines the preclinical and clinical interactions between vaccine-mediated tumor-specific immune responses and local radiation, systemic chemotherapy, or select small-molecule inhibitors, as well as the potential synergy between these modalities.

INTRODUCTION

While there have been remarkable advancements in cancer treatment over the past few years, with the advent of new therapies, the goals of reducing disease burden and improving quality of life are only sometime achieved. As some cancer vaccines demonstrate clinical activity, they will likely be used earlier in the disease process. This will require the
development of strategies to employ cancer vaccines with standard-of-care therapies that modulate the immune response. There is growing evidence that a multimodality approach targeting different aspects of the immune system may yield the greatest clinical benefit.

This review focuses on the use of therapeutic cancer vaccines with conventional therapies such as radiation, chemotherapy, and small molecule inhibitors (SMIs). Various immunomodulatory effects of conventional therapies can be exploited to enhance the antitumor activity induced by vaccines (Fig. 1). For radiation therapy, these include a) upregulation of tumor antigens, costimulatory molecules, Fas, and major histocompatibility complex (MHC) moieties, which makes tumors more susceptible to immune-mediated attack; b) upregulation of cytokines, chemokines, and adhesion molecules, which enhances T-cell trafficking to the tumor site and prolongs T-cell/tumor contact; and c) downregulation of regulatory T cells (Tregs), which facilitates generation of antigen-specific T cells. Chemotherapy’s immunomodulatory effects include a) induction of immunogenic tumor-cell death, leading to activation of dendritic cells (DCs) and facilitating cross-priming and tumor-specific T-cell generation; b) upregulation of tumor antigens, adhesion molecules, antigen-processing machinery (APM) and MHC, which increases T-cell recognition and triggers T-cell killing; and c) induction of leukopenia followed by differential homeostatic peripheral expansion (HPE) that favors tumor-specific T cells. Finally, select, targeted SMIs can a) increase the number and function of tumor antigen-specific T cells and decrease the number and function of myeloid-derived suppressor cells (MDSCs) and Tregs; b) block the tumor-cell cycle and induce apoptosis; and c) inhibit neoangiogenesis, modulate hypoxia, and normalize tumor vasculature.

Given the potential immunomodulatory effects of these established cancer therapies, combining them with cancer vaccines provides an opportunity to improve patient survival and quality of life.

COMBINING RADIATION THERAPY AND IMMUNOTHERAPY

Radiation is the standard treatment for many cancer types, traditionally employed to locally eradicate tumor cells or alter tumor and/or tumor stroma architecture with either curative or palliative intent. Although local control of the primary tumor is necessary and can usually prevent metastasis, radiation generally fails to control pre-existing systemic disease, which may be present as undetectable micrometastases. Although radiation has generally been considered immunosuppressive, several recent studies have shown that radiation actually has the potential to be immunomodulatory. Radiation-induced cell death is an immunologically active process wherein dying tumor cells release tumor-associated antigens (TAAs) that can potentially be exploited to stimulate robust tumor-specific immune responses (Fig. 1). Cells undergoing radiation-induced cell death also develop distinctive changes on their plasma membranes. These changes act as danger signals to promote phagocytosis by antigen-presenting cells (APCs) such as macrophages and DCs. Certain proteins, including heat-shock proteins, calreticulin, and high-mobility group box 1 (HMGB1), have been shown to be critical danger signals.1–3 Plasma membrane expression of heat-shock proteins, which occurs following radiation, helps mark damaged cells for elimination by the immune system and facilitates antigen cross-presentation, DC maturation, and natural killer (NK) cell activation.4–7 Calreticulin is a crucial determinant of whether dying tumor cells are phagocytosed by APCs.8,9 The nuclear nonhistone protein HMGB1 binds to toll-like receptor 4 (TLR4), thereby providing a signal to DCs to initiate TLR4-dependent antigen processing. Friedman has previously described a “danger model” of immunity wherein ionizing radiation generates an inflammatory microenvironment filled with apoptotic and necrotic cells, cytokines, chemokines, inflammatory mediators, and acute-phase reactant proteins.10 This milieu of immune modulators can activate APCs and support their
processing of newly exposed TAAs. Activated APCs then migrate to the location of radiation-induced cell death, undergo maturation, and present post-radiation cellular debris and antigens to T cells.

Radiation also modulates tumor-cell phenotype and consequently increases immune recognition. Local tumor radiation induces upregulation of MHC-I, Fas/CD95, and the costimulatory molecules B7.1, intercellular adhesion molecule 1 (ICAM-1), and lymphocyte function-associated antigen 3 (LFA-3). MHC-I is responsible for direct presentation of tumor-antigen peptides to cytotoxic T lymphocytes (CTLs), while increases in adhesion molecules improve cell-to-cell attachment and thus enhance T cells’ ability to kill target cells. Fas, a member of the tumor necrosis factor receptor family, is a death receptor that induces apoptosis upon binding to Fas-ligand. Fas-ligand displays a complex pattern of inducible and constitutive expression associated with a number of functions as a death factor and costimulatory molecule in lymphocyte activation. Activated CTLs express cell-surface Fas ligand, which binds to Fas molecules on the target cell surface, giving the target cell the signal to undergo apoptosis. Fas-mediated apoptosis has been shown to play an important role in CTL-mediated tumor cell destruction in addition to granzyme-dependent killing.

Garnett et al. demonstrated that radiation is able to alter the cell-surface expression of a variety of immunomodulatory molecules such as Fas, ICAM-1, MHC-I, and TAAs such as carcinoembryonic antigen (CEA) and mucin-1 (MUC-1). They examined 23 human carcinoma cell lines (12 colon, 7 lung, 4 prostate) for responses to nonlethal doses of radiation (10 or 20 Gy) and found that at least one of the above-named surface molecules increased in 21 of 23 (91%) cell lines studied. Furthermore, all irradiated cell lines demonstrated significantly enhanced killing compared to nonirradiated cell lines, suggesting that nonlethal doses of radiation render human tumor cells more amenable to immune recognition and attack. Microarray analysis revealed that many additional genes had been modulated by radiation. These upregulated gene products may make tumor cells even more susceptible to T cell-mediated immune attack or serve as additional targets for immunotherapy. Additionally, recent studies have indicated that radiation can affect the tumor microenvironment and tumor vasculature. These effects can facilitate homing of both APCs and effector T cells through changes in extracellular matrix proteins and adhesion molecules on endothelial cells and radiation-induced inflammatory signals.

Interest in combining radiation and immune-based therapies for the treatment of cancer is growing in proportion to our understanding of the immunomodulatory effects of radiation and radiation’s effect on tissues. A great deal of preclinical research into combining radiation plus active therapeutic cancer vaccines has been translated into clinical studies of this combination as a multimodal therapy for cancer (Table 1 & 2).

**External Beam Radiation**

Chakraborty et al. examined the combination of low-dose external beam radiation therapy (EBRT) and active therapeutic vaccination for the treatment of subcutaneous (s.c.) tumors in a mouse model. After radiation with 8 Gy, CEA+ tumor cells demonstrated an upregulation of Fas that was maintained for > 11 days (Fig. 2A). A vaccine consisting of recombinant vaccinia (rV) and recombinant fowlpox (rF) vectors expressing CEA and a triad of costimulatory molecules (B7.1, ICAM-1, and LFA-3), designated rV/rF-CEA/TRICOM, was used in this study. CEA+ murine colon carcinoma cells (MC38-CEA+) were implanted s.c. into mice transgenic for human CEA (CEA-Tg). After 8 days, mice were randomized to receive no treatment, radiation alone, vaccine alone, or a combination of radiation and vaccine (Fig. 2B). All untreated mice succumbed to progressive tumor growth by day 30. Neither radiation alone nor vaccine alone improved survival. However, the
combination was curative in > 50% of mice while also imparting protection from subsequent tumor challenge. Interestingly, mice cured of their tumors demonstrated “antigen cascade,” a term that describes the development of CD4+ and CD8+ T-cell responses to tumor antigens not encoded in the vaccine (in this case, p53 and gp70).

Results from these preclinical studies provided the rationale for clinical evaluation of the combination of EBRT and therapeutic cancer vaccines. A recent clinical trial assessed the use of a recombinant poxviral-based vaccine expressing prostate specific antigen (PSA) combined with standard definitive radiotherapy in patients with localized prostate cancer.26 Results from this clinical trial indicated that the combination was safe, well tolerated, and, more importantly, effective at generating a PSA-specific immune response. Approximately 76.5% of patients in the combination therapy arm showed a ≥3-fold increase in PSA-specific T cells vs. 0% in the radiation-alone arm (P < 0.0005).26 Another ongoing clinical trial is combining the rV/F-CEA/TRICOM vaccine with low-dose EBRT delivered directly to liver metastases in patients with CEA+ solid tumors.27

Bone-Seeking Radionuclide

In advanced stages, many primary human carcinomas such as thyroid, breast, kidney, prostate, and multiple myeloma typically involve painful bone metastases that require palliative therapy. Strontium-89 and Samarium-153 (153Sm-EDTMP; Quadramet®, Cytogen) are bone-seeking radiopharmaceuticals used to relieve the pain of bone metastases. Even though both agents are approved by the U.S. Food and Drug Administration (FDA), 153Sm-EDTMP is a superior choice for combination therapy with cancer vaccines due to its shorter half-life, which allows for repeated administration and faster recovery from treatment-induced pancytopenia. The dose of palliative radiation delivered to bone metastases by 153Sm-EDTMP is calculated to be between 18 and 80 Gy.28, 29 As noted, these doses are associated with phenotypic modulation of human tumor cells. One study demonstrated that of 10 human tumor-cell lines representing tumors that metastasize to bone (4 prostate, 2 breast, and 4 lung), 100% upregulated Fas and CEA, 70% upregulated MUC-1, 40% upregulated MHC-I, and 30% upregulated ICAM-1 when exposed to clinically relevant palliative levels of 153Sm-EDTMP for 4 days. Exposure of LNCaP cells (prostate cancer) to 153Sm-EDTMP also resulted in upregulation of tumor antigens such as PSA, prostate specific membrane antigen, prostatic acid phosphatase, CEA, and MUC-1. Upregulation of these tumor antigens rendered the cells more susceptible to lysis by CTLs specific for PSA, CEA, and MUC-1, suggesting that 153Sm-EDTMP works synergistically with immunotherapy.

The combination of 153Sm-EDTMP and vaccine is currently being studied in a randomized phase II study at the National Cancer Institute, designed to determine if 153Sm-EDTMP combined with vaccine can improve time to progression over 153Sm-EDTMP alone in patients with castration-resistant prostate cancer (CRPC) metastatic to bone.30 Patients will receive 153Sm-EDTMP alone or in combination with an rV/rF-PSA/TRICOM vaccine (PROSTVAC®, Bavarian Nordic) administered in a diversified prime/boost regimen.

Radiolabeled Monoclonal Antibodies

Therapeutic radionuclides can be delivered systemically to cancer cells via monoclonal antibodies (mAb). This method precisely and preferentially targets tumor cells and seeks out micrometastases unobservable by current imaging technology and thus insusceptible to EBRT. A recent report cited radiolabeled mAb’s ability to alter tumor-cell phenotype and enhance immunologic targeting of tumor cells, as well as a therapeutic synergy between radiolabeled mAb and cancer immunotherapy.31 This study employed an yttrium-90 (Y-90)-labeled anti-CEA mAb either alone or in combination with a CEA-targeted vaccine to treat
mice implanted with CEA+ murine carcinoma cells. The combination of vaccine and a single dose of Y-90-labeled anti-CEA mAb resulted in a statistically significant increase in survival of tumor-bearing mice over either modality alone. Additionally, the combination group exhibited a significant increase in the percentage of viable tumor-infiltrating CEA-specific CD8+ T cells compared to the vaccine alone group. Surprisingly, the tumor-infiltrating T cells were unaffected by the residential radiation being emitted by the radiolabeled mAb. This finding was consistent with a preclinical study by Grayson et al. which found that murine memory T cells are more resistant to apoptosis than naïve T cells after whole-body irradiation. Mice cured of tumors also demonstrated an antigen cascade, as seen with EBRT.32

Brachytherapy

Brachytherapy involves implanting a radiation source into or near the site of a malignant tumor to target tumor cells with continuous high-dose radiation. A single study reported the ability of iodine-125 and a recombinant poxviral vaccine to modulate tumor cell phenotype and enhance antigen-specific killing of tumor cells.33 While more in-depth studies are needed to validate these results, they do suggest a clinical role for the combination of brachytherapy and cancer vaccines.

In summary, a growing body of evidence suggests that an appropriate dose of radiation can have immunomodulatory effects capable of activating the immune system and subsequently enhancing immune-mediated attack on tumor cells. Many preclinical studies have demonstrated that radiotherapy and cancer vaccines combined work synergistically to generate more robust antitumor effects.1, 13, 17, 18, 31, 34 Promising results from these preclinical studies have led to several clinical trials (Table 2). As the field of cancer therapy advances, monotherapies may fall into disfavor. In fact, many preclinical and clinical studies have combined more than 2 therapeutic modalities. One murine study combined vaccine, local radiation, and reduction of immune suppressor cells,35 while an in vitro study reported the combination of systemic multiagent chemotherapy with 5-fluorouracil (5-FU) and cisplatin with tumor irradiation for the treatment of head/neck squamous cell carcinoma (HNSCC).36

COMBINING CHEMOTHERAPY AND IMMUNOTHERAPY

The clinical efficacy of standard-of-care chemotherapy regimens relies mostly on direct cytotoxicity to cancer cells. Until recently, it was generally believed that when used in combination with a cancer vaccine, chemotherapy would invariably have a negative effect on vaccine-mediated immune responses and antitumor activity.37 However, mounting evidence suggests that certain chemotherapeutic agents have immunomodulatory properties that can be exploited to enhance vaccine-mediated antitumor effects (Fig. 1). This synergy can be mediated by multiple mechanisms, depending on the type of cytotoxic agent and the specific vaccine employed, as well as the dosing schedule of each modality.

Chemotherapeutic agents can modulate the phenotype of tumor cells by altering the expression of TAAs, MHC-I, ICAM-1, and APM, making them more susceptible to immune-mediated attack.36, 38, 39 These agents can also induce “immunogenic death” of tumor cells, leading to IL-12-mediated activation of DCs, followed by antigen presentation and cross-presentation to T cells, resulting in CTLs with greater and more efficient cytotoxic potential.40–42 In addition, cytotoxic agents can have direct effects on the host immune system, including a) modulation of immune regulatory elements such as Tregs and MDSCs,43–45 b) induction of leukopenia followed by differential HPE of regulatory and effector immune subsets,46–48 and c) synergy with vaccine to increase effector immune responses to multiple TAAs.49
Recent evidence also suggests that specific chemotherapeutic regimens can reduce the tumor growth rate in cancer patients when combined with certain cancer vaccines. Detailed reviews of the synergistic effects of cancer chemotherapy and immunotherapy regimens have previously been published. Many preclinical studies have explored combinations of mature vaccine platforms with chemotherapy, some of which have been translated into the clinic (Table 1 & 2).

**Platinum Alkylating Agents: Oxaliplatin, Cisplatin, Cisplatin/5-FU, and Cisplatin Plus Vinorelbine**

Platinum alkylating agents such as oxaliplatin and cisplatin are commonly used to treat a variety of malignancies, including non-small cell lung cancer (NSCLC) and HNSCC. The cytotoxicity of these agents is rendered through DNA crosslinking. However, accumulating evidence suggests that nontoxic concentrations of these agents can induce immune-relevant changes in tumor cells and several components of the immune system. These alterations could be exploited in a combined chemotherapy/vaccine regimen to achieve potent antitumor immunity. In one study, tumor cells exposed to oxaliplatin expressed higher levels of MHC-I proteins and secreted cytokines able to augment DC maturation, resulting in the generation of CTLs with increased cytotoxic potential. Cisplatin has also been shown to modulate tumor cell characteristics toward a more immunogenic phenotype. Exposure to nontoxic levels of cisplatin increased expression of functional Fas receptor on murine tumor cells, leading to augmented CTL-mediated lysis. Increased sensitivity to antigen-specific CTLs was also observed in human colon carcinoma cell lines treated with cisplatin, an effect associated with enhanced expression of ICAM-1 and Fas. Similar results have been reported with chemotherapy combinations including cisplatin. In one study, exposure of HNSCC cell lines to cisplatin plus 5-FU resulted in a synergistic increase of ICAM-1. Concurrent exposure of Lewis lung tumor cells to sublethal concentrations of cisplatin plus vinorelbine was shown to modulate expression of survival genes and increase expression of Fas and MHC-I molecules, resulting in increased sensitivity to CTL-mediated lysis (Fig. 3A). These immune-relevant changes translated into decreased resistance to Fas-dependent lysis by CTLs. In other preclinical studies, the addition of cisplatin augmented antitumor immunity elicited by viral, DNA, and subunit vaccine platforms.

Chemotherapeutic regimens often result in moderate to severe lymphopenia, an adverse event considered detrimental to the generation of effective antitumor immunity. However, recent reports focusing on the mechanisms of HPE of immune subsets following iatrogenic leukopenia suggest that this period of T cell reconstitution presents a unique opportunity to expand vaccine-mediated antitumor immunity. A recent study combining a yeast-CEA vaccine with chemotherapy demonstrated that cisplatin plus vinorelbine differentially modulates the HPE of effector and regulatory T cell subsets (Fig. 3B) and synergizes with vaccine, resulting in enhanced CEA-specific immune responses (Fig. 3C). Moreover, in a preclinical murine model of established NSCLC, the combination of this chemotherapeutic doublet with vaccine increased survival of tumor-bearing mice, an effect mediated by both CD4+ and CD8+ T cell subsets (Fig. 3D). Cisplatin plus vinorelbine has been clinically evaluated in combination with a recombinant modified vaccinia Ankara (MVA) vaccine expressing MUC-1 and IL-2 (TG4010).

**Taxanes: Paclitaxel and Docetaxel**

Taxanes are among the most widely used cancer chemotherapeutic agents and have been employed to treat a variety of malignancies, such as breast, prostate, and lung carcinomas. In addition to the well-described cytotoxic effects of taxanes, elicited through microtubule disruption, nontoxic concentrations can induce an immunogenic phenotype in tumor cells that is more amenable to immune-mediated lysis. For example, exposure of human colon
carcinoma cells to nontoxic concentrations of paclitaxel has been shown to upregulate the expression of APM proteins, including calmodulin, low molecular mass polypeptide 2 and 7, transporter 1, and tapasin, suggesting the potential for increased recognition by CTLs.\textsuperscript{38} Similarly, exposure of human ovarian carcinoma cells to sublethal concentrations of paclitaxel induced enhanced NK cell-mediated lysis mediated by increased ICAM-1 expression.\textsuperscript{67}

In addition to their direct effects on tumor immunogenicity, taxanes can modulate various elements of the host immune system, including increasing macrophage activation and inducing intratumoral release of inflammatory cytokines, resulting in augmented tumor lysis.\textsuperscript{68} Sublethal concentrations of paclitaxel have been shown to enhance IL-12-dependent antigen presentation by DCs, an effect associated with increased expression of APM components and enhanced costimulation.\textsuperscript{41} Further, it has been reported that exposing DCs to paclitaxel-pretreated tumor cells generates CD8\textsuperscript{+} T cells with higher lytic potential.\textsuperscript{38}

Accumulating evidence suggests that the immediate period of T cell reconstitution following chemotherapy-associated lymphopenia offers a unique opportunity to expand effective antitumor immunotherapy.\textsuperscript{46, 49, 64, 65} For example, docetaxel has been reported to modulate T cell, B cell, and NK cell subsets and to enhance CD8\textsuperscript{+} function while deleting Tregs.\textsuperscript{49} In preclinical studies in CEA-Tg mice transplanted with CEA\textsuperscript{+} tumor cells, antitumor responses were enhanced by a combination of docetaxel and an rV/F-CEA/TRICOM vaccine regimen, compared to responses induced by vaccine or docetaxel alone (Fig. 4A and B). Docetaxel administered after vaccination optimally enhanced immune responses to the recombinant viral vaccines, including antigen-specific T cell responses to the TAA delivered by the vaccine, as well as to cascade antigens derived by the tumor (Fig. 4C).\textsuperscript{49} Docetaxel has been or is currently being evaluated in combination with a) an rV vaccine expressing PSA and B7.1 (rV-PSA/B7.1; PROSTVAC\textsuperscript{®}) b) a diversified prime/boost vaccine using vaccinia and fowlpox viruses expressing MUC-1, CEA, and TRICOM (PANVAC\textsuperscript{®}), and c) the DC vaccine Provenge\textsuperscript{®} (Dendreon Corp.), among other cancer vaccine platforms.

**Alkylating Agents: Cyclophosphamide and Combinations**

Alkylating agents like cyclophosphamide are included in chemotherapy regimens for a wide range of malignancies. In addition to their direct cytotoxic effect on tumor cells through DNA alkylation, these agents have immunomodulatory properties that can be exploited in a therapeutic regimen that includes a cancer vaccine. Cyclophosphamide has been shown to increase human leukocyte antigen expression and cytokine secretion in tumor cells, leading to increased maturation of DCs and augmented CTL killing.\textsuperscript{52} Direct effects of cyclophosphamide on DCs and other elements of the host immune system are well documented.\textsuperscript{42, 44} For instance, CD8\textsuperscript{+} T cells exposed to cyclophosphamide have increased lytic function.\textsuperscript{69}

Accumulating evidence indicates that Tregs play a crucial role in T cell tolerance of tumors, constituting a major barrier to the generation of effective antitumor immunity in carcinoma patients.\textsuperscript{70, 71} Cyclophosphamide has been shown to abrogate the suppressive influence of Tregs, allowing the activation of potent, vaccine-mediated immunity.\textsuperscript{43–45} In an experimental melanoma model, systemic cyclophosphamide combined with a DC vaccine led to improved antitumor effects.\textsuperscript{72} Metronomic doses of cyclophosphamide have been evaluated clinically in combination with a sialyl-Tn-keyhole limpet hemocyanin vaccine (THERATOPE\textsuperscript{®}, Biomira) for the treatment of metastatic breast cancer.\textsuperscript{73}

In another example, proper timing of cyclophosphamide, doxorubicin, and paclitaxel treatment enhanced the antitumor immune response to a whole tumor cell vaccine in a preclinical model of breast carcinoma.\textsuperscript{45} In this case, the augmented antitumor effects of the
combination of vaccine plus chemotherapy were due to enhanced vaccine efficacy rather than the direct cytotoxic effect of chemotherapy on cancer cells. The combination of cyclophosphamide and doxorubicin was recently evaluated in combination with a GM-CSF-secreting HER2/neu-expressing whole tumor cell vaccine in patients with metastatic breast cancer.74

**Anthracyclines: Doxorubicin**

Anthracyclines such as doxorubicin are DNA-intercalating agents used to treat a wide range of malignancies, including carcinomas of the breast, ovary, bladder, and lung. Cancer cells exposed to cytotoxic concentrations of doxorubicin undergo rapid translocation of ERp57 and calreticulin to the cell surface, triggering caspase-dependent immunogenic cell death, an effect not observed with other DNA-damaging agents.40, 75 Noncytotoxic concentrations of doxorubicin enhance IL-12-dependent antigen presentation by DCs, an effect associated with modulation of APM components, leading to increased effector T cell function.41

**Antimetabolites: Gemcitabine, Methotrexate, and 5-FU**

Antimetabolites are indicated for the treatment of several malignancies, including carcinomas of the pancreas and colon, and HNSCC. Various immunomodulatory properties of these agents have been revealed. For example, gemcitabine can upregulate MHC-I on tumor cells, resulting in enhanced sensitivity to CTL-mediated lysis.52 Similarly, treatment of human colon carcinoma cell lines with 5-FU can enhance their sensitivity to the cytotoxic effects of CD8+ T cells by inducing expression of ICAM-1 and Fas.56 Direct effects of antineoplastic agents such as methotrexate on T cell cytotoxicity have also been reported.69

Antimetabolites can enhance DC function by direct and indirect mechanisms. In one report, direct exposure of DCs to methotrexate resulted in increased antigen presentation to T cells.41 Stimulation of DC function has been associated with upregulation of IL-12 and APM elements, and augmented DC function has been seen after exposure to tumor cells treated with gemcitabine.52 It has also been demonstrated that gemcitabine can selectively reduce MDSCs in tumor-bearing mice without affecting other immune cell populations.76

**Chemotherapy Plus Radiation**

Cisplatin plus 5-FU chemotherapy, combined with tumor irradiation, is the standard of care for HNSCC. The combination of chemotherapy and radiation has been shown to significantly decrease Bcl-2 and increase the sensitivity of human HNSCC target cells to perforin-mediated, MHC-restricted CTL killing, compared to target cells exposed to either modality alone.56 The studies described in this review highlight the rational basis for the clinical combination of immunotherapy and the current standard of care for HNSCC.

**Other Potential Combinations**

The immunomodulatory functions of many other chemotherapeutic agents are currently being investigated, for potential use in combination with therapeutic cancer vaccines. For example, lenalidomide, a chemotherapeutic agent FDA-approved for the treatment of multiple myeloma, has been shown to have several immunomodulatory effects, including augmentation of T cell function, stimulation of NK cell cytotoxicity, and suppression of Treg function and proliferation.77, 78 Like other agents of its class, lenalidomide is also antiangiogenic and antiapoptotic, and can reduce the metastatic capacity of tumors.79, 80

There is increasing interest in the potential therapeutic benefits of regimens combining cancer vaccines plus standard-of-care chemotherapy. However, there are several important considerations. First, employing vaccine and chemotherapy early in the disease process can have significantly different clinical outcomes than administering vaccine after multiple
chemotherapeutic regimens in advanced-stage disease, when the immune system is most likely impaired. Second, not all chemotherapeutic agents are compatible with vaccine. And third, when used with chemotherapy, the timing of vaccine administration may be extremely important. Accumulating preclinical evidence of the immunomodulatory effects of chemotherapy presents new options for combining chemotherapy with vaccine to generate effective antitumor immunity in the clinical setting. Several mature platforms are already in use clinically (Table 1). Further clinical studies will be required to optimize the use of these and other combination regimens.

COMBINING SMALL MOLECULE INHIBITORS AND IMMUNOTHERAPY

In the last decade, use of targeted SMIs for the treatment of many tumor types has increased. The major difference between standard chemotherapeutic agents and SMIs is that the former suppress rapidly proliferating cells while the latter target specific protein-protein interactions, such as growth factors and their receptors. Compared to standard chemotherapy, targeted therapy with SMIs has the advantage of modulating specific cellular pathways that are crucial for tumor biology, along with the benefits of decreased toxicity and increased effectiveness.

There are also many potential benefits of combining SMIs with immunotherapy. Some SMIs can selectively increase immune activation (Fig. 1) by inhibiting immune suppressor cells such as Tregs and MDSCs and/or by activating immune effector cells such as CTLs and DCs. SMIs can make tumor cells more susceptible to immune-mediated killing by improving tumor-specific antigen presentation and/or FAS-mediated killing. Also, the synergistic effect of combining SMIs with vaccine can justify the administration of SMIs at a lower dose, further decreasing the potential for toxicity. Achieving an optimal outcome when combining immunotherapy and SMIs requires determining the appropriate timing of SMI treatment and vaccine administration. The best combination schedule should result in robust immune stimulation against TAAs, with little or no toxicity against immune effector cells.

Bcl-2 Inhibitors

One class of SMIs inhibits Bcl-2 molecules. SMIs that alter the balance between pro- and antiapoptotic Bcl-2 family members have shown potential benefit in preclinical cancer models. The Bcl-2 inhibitors ABT-737 and GX15-070, currently being tested as cancer therapeutics, act by mimicking the proapoptotic BH3 domain in order to induce apoptosis in cancer cells. ABT-737 targets Bcl-2 and Bcl-2-related proteins such as Bcl-xL and Bcl-w, but not A1 or Mcl-1, which may prove valuable in treating lymphoma and other blood cancers as well as solid tumors. When peptide-pulsed DC vaccination was given both prior to and after tumor implantation, ABT-737 administration increased the antitumor activity of vaccination in a CT26 colon carcinoma model (Table 1). ABT-737 is currently being evaluated in advanced phase clinical trials.

GX15-070, a pan-Bcl-2 inhibitor, is a synthetic derivative of bacterial prodiginines. GX15-070, which has the ability to bind all antiapoptotic Bcl-2 family members, including Bcl-2, Bcl-xL, Bcl-w, Mcl-1, and BAK, induces apoptosis in hematologic and solid tumor cells in vitro and in vivo and is currently being investigated in clinical trials. The effect of GX15-070 on CD8+ T cells is dependent on their activation status (Fig. 5A). Upregulation of the Mcl-1 gene has been reported within 10 h of T cell receptor ligation, indicating that Mcl-1 is involved in early T cell activation. The fact that GX15-070 inhibits Mcl-1 ligation to the proapoptotic BAK could explain why early-activated lymphocytes are more sensitive to the inhibitor (Fig. 5B). Mature CD8+ lymphocytes, which are resistant to GX15-070, display increased binding of the proapoptotic BAK to the
antiapoptotic Mcl-1 (Fig. 5B). These data suggest that if vaccination were to precede GX15-070 treatment by an interval sufficient to overcome early activation, vaccine-induced T cells would not be negatively affected by the inhibitor. Furthermore, the proliferation of CD8+ T cells was significantly higher when they were cocultured with Tregs from GX15-070-treated mice than when they were cocultured with Tregs from untreated mice, indicating that GX15-070 inhibits Treg function (Fig. 5C). This suggests that GX15-070 can mediate an increase in immune-mediated antitumor activity by decreasing Treg-dependent immune suppression. This effect, along with an increased intratumoral activated CD8:Treg ratio in mice first vaccinated with rV/F-CEA/TRICOM then treated with the inhibitor, suggests that such a combination can produce a favorable milieu for immune activity against tumor cells. Sequential therapy with this vaccine followed by GX15-070 effectively reduced orthotopic pulmonary tumors in immunocompetent mice (Fig. 5D), suggesting a rationale for the design of similar combination protocols for clinical studies.

The studies described above indicate that when combining SMIs with immunotherapy, the appropriate interval between administration of each agent is important. Vaccine-induced immunity may be reduced when the Bcl-2 inhibitor is administered concurrently with or shortly after vaccine, since early-activated lymphocytes are extremely sensitive to GX15-070. Thus, in a combination setting, it is important that vaccine be administered long enough before GX15-070 to allow activated lymphocytes to mature.

**Tyrosine Kinase Inhibitors**

Another promising and intensely studied class of SMIs that could be used in combination with immunotherapy is tyrosine kinase inhibitors (TKIs). Approximately 30 kinase targets are being developed to the level of clinical trial, the vast majority of which are being investigated for the treatment of cancer. To date, approximately 80 TKIs have advanced to some stage of clinical evaluation and 11 have received FDA approval for cancer treatment, possibly because many tyrosine kinases have been found to be integral to the processes leading to tumor cell proliferation and survival.

Sunitinib and sorafenib are members of a class of TKIs that inhibit tumor vasculature. Sunitinib, an orally available inhibitor of multiple TKIs, was approved by the FDA in 2006 for the treatment of advanced renal cell carcinoma (RCC) and imatinib-resistant gastrointestinal stromal tumors. Sunitinib is currently being evaluated as a treatment for many other solid and hematologic malignancies in numerous clinical trials, including nearly 150 studies sponsored by the National Cancer Institute.

Tyrosine kinase receptors targeted by sunitinib, such as receptors for vascular endothelial growth factor (VEGF) and platelet-derived growth factor, are widely expressed in many tumor cell types and tumor vasculature, allowing sunitinib to act directly against tumor cells and tumor stroma. Sunitinib also targets tyrosine kinase receptors expressed on MDSCs, such as c-KIT and VEGFR-1, making it a promising immunomodulatory. In fact, sunitinib exerts powerful immunomodulatory effects in cancer patients, such as shifting Th2 immune responses to Th1 and inhibiting immune suppressor cells, making this TKI an attractive candidate for combination with immunotherapies.

A recent preclinical study investigated the immunomodulatory effects of sunitinib in order to support the rational design of clinical trials combining sunitinib with immunotherapeutic platforms for the treatment of solid tumors. Using a mouse model, this study investigated the effects of sunitinib given for 4 weeks at concentrations comparable to 37.5 to 50 mg/day in humans, followed by 2 weeks off (sunitinib 4/2). In vivo, one cycle of sunitinib 4/2 caused bimodal immune effects: a decrease in regulatory cells during the 4 weeks of treatment, followed by an immune-suppression rebound during the 2 weeks of treatment.
interruption. A regimen of sunitinib followed by vaccine caused increased proliferation of antigen-specific CD4+ T cells (Fig. 6A) and increased numbers of antigen-specific CD8+ T cells (Fig. 6B). In contrast, coadministration resulted in a transient decrease of T lymphocytes at day 2 following sunitinib treatment, suggesting that giving vaccine at the initiation of sunitinib treatment could compromise the vaccine-induced immune response. In CEA-Tg mice bearing CEA+ tumors, continuous sunitinib treatment followed by vaccine increased intratumoral infiltration of antigen-specific T cells, decreased Tregs and MDSCs, reduced tumor volume, and increased survival (Fig. 6C). These data indicate that a) the immunomodulatory activity of continuous sunitinib can create a more immune-permissive environment, and b) in combination with immunotherapy, sunitinib should precede vaccine in order to precondition the immune system and maximize the response to vaccine-mediated immune enhancement. A recent randomized phase III clinical study combining MVA encoding the TAA 5T4 (MVA-5T4; TroVax®, Oxford BioMedica) with sunitinib in RCC showed no difference in survival between patients receiving sunitinib alone and patients receiving sunitinib with vaccine.104 However, in this trial patients were vaccinated prior to receiving sunitinib, which, as indicated above, may not be the most appropriate regimen.

Clinical translation of combinatorial therapies involving SMIs and vaccines must take into consideration the particular effects of the SMI on immune cells. Studies have indicated that an SMI that selectively inhibits immune suppressor cells (i.e., sunitinib) should be administered prior to vaccine in order to enhance the vaccine-mediated immune response to TAAs. If, on the other hand, the SMI alters lymphocyte activation (i.e., GX15-070), vaccinating before SMI treatment and allowing sufficient time for the activated lymphocytes to mature should result in more resistance to toxicity. Finally, if the SMI does not affect activation of effector lymphocytes and does not inhibit immune suppressors, it can be coadministered with immunotherapy.

SYNERGY

Taken together, the results from the preclinical and clinical studies described herein demonstrate the rationale for, and potential advantages of, combining therapeutic cancer vaccines with radiation, chemotherapy, or SMIs therapy. Each modality affects a different part of the immune system and tumor biology, potentially enhancing the action of the other modalities.

Cancer chemotherapy began in the 1940s with only nitrogen mustards and evolved to include combinations of multiple classes of chemotherapy agents targeting distinct factors of tumor growth (i.e., FOLFOX, FOLFIRI). Currently the same evolution is occurring in the field of small molecule inhibitors with the approval of Gleavec, bevacizumab, vandetanib, and gefitinib just to name a few. We envision combination immunotherapy evolving in a similar way, from vaccines as monotherapy, to vaccines combined with standard-of-care radiation, chemotherapy, and small-molecule therapeutics, to novel experimental therapies. As each standard modality has unique features that can enhance vaccine efficacy, it is conceivable that a multimodal approach encompassing several therapy platforms in combination with vaccines could result in even greater synergistic antitumor effects.

Acknowledgments

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References


Figure 1.
Multiple mechanisms of synergy between radiation therapy, chemotherapy, or small molecule inhibitors and immunotherapy.
Figure 2.
External beam radiation altered the phenotype of tumor cells and enhanced tumor-cell lysis when combined with vaccine. (A) Radiation-induced upregulation of Fas on tumor cells from s.c. MC38-CEA\(^+\) tumor-bearing mice was maintained for > 11 days post-irradiation. To confirm Fas expression, tumors were immunostained with anti-Fas mAb. (B) Used alone, neither vaccine nor radiation effectively reduced tumor volume, while the combination was curative in a majority of tumor-bearing mice. The combination also conferred protection from subsequent tumor challenge (data not shown). Adapted from 17.
Figure 3.
When combined with vaccine, chemotherapy altered tumor-cell phenotype, enhanced vaccine-mediated T-cell responses, and improved survival of tumor-bearing mice. (A) Treatment with cisplatin/vinorelbine increased Fas cell-surface expression and sensitivity to CTL-mediated killing of Lewis lung carcinoma cells 48 h post-exposure \textit{in vitro}. (B) Cisplatin/vinorelbine reduced the homeostatic peripheral expansion of Tregs for >8 days in C57BL/6 mice, while CD8\(^+\) T cell expansion recovered by day 4 post-chemotherapy. (C) Combining cisplatin/vinorelbine with a yeast-CEA vaccine improved CD4\(^+\) T cell proliferation and CEA-specific T-cell responses. (D) The combination of cisplatin/vinorelbine with yeast-CEA increased survival in a murine model of established NSCLC. Adapted from\(^{46}\).
Figure 4

Docetaxel treatment improved vaccine-mediated immune and antitumor responses. (A) CEA-specific murine CD4+ T-cell responses improved when docetaxel was administered after rV/F-CEA/TRICOM vaccine. (B) Docetaxel significantly increased the antitumor activity of vaccine in CEA-Tg mice bearing s.c. MC38-CEA+ tumors. (C) The CD8+ cascade response to gp70 improved following vaccine plus docetaxel treatment in MC38-CEA+ tumor-bearing mice. Adapted from.49
Vaccinating prior to treatment with a Bcl-2 inhibitor (GX15-070) increased CD8\(^+\) resistance to apoptosis, diminished Treg suppression, and decreased pulmonary tumors. (A) Mature CD8\(^+\) T lymphocytes were more resistant to increasing concentrations of GX15-070 than early-activated lymphocytes after 72 h of treatment in vitro. (B) Mature CD8\(^+\) T lymphocytes exhibited increased binding of the proapoptotic BAK to the antiapoptotic Mcl-1 after GX15-070 treatment. (C) In C57BL/6 mice, treatment with GX15-070 reduced the suppressive activity of splenic Tregs. (D) Vaccination followed by GX15-070 treatment led to significantly fewer pulmonary metastases in a model of Lewis lung carcinoma.

Adapted from. 93
Figure 6.
Vaccinating after sunitinib treatment increased tumor-specific immunity and improved survival in tumor-bearing mice. (A) In CEA-Tg mice, sunitinib followed by vaccine increased proliferation of CEA-specific CD4+ T cells. (B) The number of CEA tetramer+ CD8+ T cells also increased with this combination. (C) In mice bearing s.c. MC38-CEA+ tumors, the sequential combination improved survival compared to single control, single therapies, or different combination timings (data not shown). * = P < 0.05; *** = P < 0.001. Adapted from.103
Table 1

Preclinical studies of combination therapy.

<table>
<thead>
<tr>
<th>Agent</th>
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<th>Model</th>
<th>Results</th>
<th>Refs</th>
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<td>Yeast-CEA</td>
<td>mLLC-CEA metastatic Lewis lung carcinoma model</td>
<td>Increased survival</td>
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<td>Cyclophosphamide, doxorubicin, paclitaxel</td>
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<td>MCA26 colon carcinoma model</td>
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<td>MC38-CEA colon carcinoma model</td>
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Clinical studies of combination therapy.

### Table 2

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EBRT = external beam radiation therapy; CRPC = castration-resistant prostate cancer; NSCLC = non-small cell lung cancer