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Chemoimmunotherapy

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Abstract

Cancer chemotherapy drugs are historically regarded as detrimental to immunity due to their myelosuppressive effects. However, accumulating data suggest that the antitumor activity of conventional cancer chemotherapy results in part from its ability to harness the innate and adaptive immune systems by inducing immunologically active tumor cell death. Additional data broaden the immunologic impact of cancer chemotherapy drugs, demonstrating that some drugs have the ability to disrupt pathways of immune suppression and immune tolerance in a manner that depends on the drug dose, and the timing of its administration in relation to immunotherapy. Understanding the cellular and molecular basis of the interactions between chemotherapy drugs and the immune system will facilitate the strategic development of chemoimmunotherapy treatment regimens that both maximize tumor regression and the antitumor immune response for the long term clinical benefit of cancer patients.

Keywords

immunotherapy; chemotherapy; chemoimmunotherapy; cancer vaccines; adoptive cellular therapy; clinical trials

Introduction

Cancer therapy has evolved to strategically integrate distinct treatment modalities in order to optimize the chance of cure. Surgery and radiation therapy are used to achieve locoregional control, whereas systemic therapies (chemotherapy, endocrine therapy, molecularly targeted therapies, and adjunctive therapies (bisphosphonates)) are used to control diffuse disease (in hematologic malignancies) or disease that has spread beyond the primary site (in solid tumors). Multiple drugs with complementary mechanisms of action and non-overlapping toxicities are frequently combined for additive or synergistic antitumor efficacy. Cancer

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Conflict of Interest Statement

This review describes work using granulocyte-macrophage colony-stimulating factor-secreting tumor vaccines. Under a licensing agreement between Biosante, Incorporated and the Johns Hopkins University, the University is entitled to a share of royalty received by the University on sales of products described in this article. The terms of this agreement are being managed by the Johns Hopkins University in accordance with its conflict of interest policies.

chemotherapy drugs typically disrupt fundamental regulatory pathways essential for tumor cell growth and survival, but are too often limited by both primary and acquired mechanisms of drug resistance and collateral toxic damage to normal host tissues. In addition, their use historically fails to consider the potential impact of systemic therapies on the overall tumor microenvironment established by the host-tumor interaction. These fundamental limitations to the traditional use of chemotherapy for systemic disease control calls for a radical but strategic shift in the way tumors are treated in order to achieve better clinical outcomes.

More recently, the host-tumor interaction has definitively emerged as a major determinant of clinical course and treatment outcomes for human malignancies[1–3]. Elements of the host response to cellular transformation critical for tumor growth and progression include stromal fibroblasts, host endothelial cells, tumor-associated macrophages (TAMs), natural killer (NK) cells, and tumor-specific lymphocytes. Multiple drugs in common use manipulate the host-tumor interaction to favor tumor regression. Bevacizumab, sunitinib, and sorafenib are used to target endothelial cell biology and disrupt the tumor-associated vasculature[4]. Multiple monoclonal antibodies uniquely marry the specificity of tumor cell-targeted therapy and immunomodulation by specifically binding to tumor cells, then recruiting host innate immune effectors (NK cells and monocytes) to mediate tumor cell cytotoxicity[5]. Cancer vaccines can actively induce tumor-specific immune responses by eliciting both a humoral (antibody) and a cellular (T cell) immune response[6]. Advantages of the therapeutic antigen-specific host immune response compared to traditional cancer treatments include exquisite tumor cell specificity, minimal off-target side effects, and the potential for a durable, lasting treatment effect in the absence of continuous, active therapy due to immunologic memory. The first personalized therapeutic cancer vaccine, sipuleucel-T (Provenge), was recently approved by the US Food and Drug Administration for the treatment of advanced prostate cancer. The approval of a drug specifically designed to induce a therapeutic tumor-specific immune response marks the beginning of an era of an entirely different approach to cancer therapy.

Active immune-based approaches to cancer therapy will clearly add a new dimension to cancer care, but they are unlikely to replace traditional cancer therapies. Moreover, the complex interactions between cancer cells and host elements within the tumor microenvironment imply that targeting one aspect of tumor biology will have clear consequences for other elements involved in tumor growth and progression. Thus, chemotherapy not only kills cancer cells directly, but also impairs endothelial cells and impacts tumor immunity[7]. Anti-angiogenic therapies not only impact tumor-associated endothelial cells, but also modulate tumor immunity[8]. Tumor-specific monoclonal antibodies not only kill cancer cells directly, but also recruit immune effectors to the therapeutic effect[9]. Thoughtful consideration of the impact of established cancer drugs like chemotherapy on the vaccines designed to elicit an antitumor immune response (or adoptive cellular therapy) will be required for the most effective integration of immune-based therapy into multimodality cancer care. Current data suggest that combination chemoimmunotherapy regimens have great potential for optimizing the clinical outcomes of cancer patients.

Tumor Immunity and Chemotherapy

Pre-existing mechanisms of immune tolerance and immune escape establish a harsh landscape for effective immune-mediated tumor rejection[10]. This includes deletion of high avidity tumor-specific T cells, the accumulation of $CD4^+CD25^+FoxP3^+$ regulatory T cells (Treg) and myeloid-derived suppressor cells (MDSCs), the secretion of transforming growth factor-β (TGF-β), tumor necrosis factor (TNF), and/or interleukin 10 (IL-10)), the expression of negative co-stimulatory molecules by tumor cells, and mechanisms of immune

escape (evolution of antigen loss variants and/or MHC-negative tumor cells, or tumor cells with defects in antigen processing). Compounding this, many standard and high dose chemotherapy regimens are immunosuppressive, inducing or contributing to lymphopenia. Some of these regimens require the co-administration of glucocorticoids, which can be both directly lympholytic and immunosuppressive[11]. In addition, cancer chemotherapy frequently results in cell death by apoptosis rather than necrosis12¯14. Apoptosis is a program of cell death long thought to be immunologically inert15, which would further decrease the likelihood of effective immune-mediated tumor destruction.

In contrast, a growing body of evidence suggests that chemotherapy may augment tumor immunity by a variety of mechanisms (Table 1). Very recent evidence suggests that apoptosis, or at least a subset of it, can be highly immunogenic[16]. Supporting this concept, standard neoadjuvant therapy has been associated with the development of immune cell infiltrates[17]. Chemotherapy-induced cell death by apoptosis can enhance cross-priming, thereby increasing the antitumor T cell response[13]. Alternatively, chemotherapy can be used in distinct ways specifically for its immunomodulatory potential[18]. First, chemotherapy can condition the tumor microenvironment by modulating the expression of tumor antigens, accessory molecules of T cell activation or inhibition, and molecules involved in antigen processing and presentation. Second, chemotherapy can be used to manipulate systemic pathways of immune tolerance and regulation. These direct immune-modulating effects of chemotherapy are not only drug-dependent, but also dependent on drug dose and schedule[18]. For example, a single low dose of cyclophosphamide given 1–3 days prior to antigen exposure can overcome immune tolerance, augmenting both humoral and cellular immunity. Conversely, cyclophosphamide given concurrently with or subsequent to an antigen exposure induces immune tolerance.

Mechanisms of Chemotherapy-Induced Immunomodulation

Immunogenic Cell Death

Many chemotherapy drugs exert their therapeutic effect by the induction of apoptosis, or programmed cell death. This may occur through the enhancement of pre-existing immunity by treatment-related apoptosis. This mechanism has been demonstrated in murine models with gemcitabine, doxorubicin, cyclophosphamide, and paclitaxel *in vivo*13،19[–23] At least one study has suggested the relevance of this mechanism in humans. The neoadjuvant therapy of locally advanced breast cancer with paclitaxel resulted in the new accumulation of tumor infiltrating lymphocytes post-treatment, where the extent of T cell infiltration correlated with clinical response (0% with stable disease, 25% with partial clinical response, and 67% with complete clinical response but some residual pathologic disease)[17]. The extent of tumor cell apoptosis with the first paclitaxel treatment predicted the accumulation of TIL and clinical benefit.

For some drugs, including anthracyclines (doxorubicin, daunorubicin, and mitoxantrone) and oxaliplatin, the mechanism underlying immunogenic tumor cell death post-chemotherapy has been elucidated12،17،24. The observation that some drugs induce apoptosis that is immunogenic and others induce apoptosis that is not suggests that alternate pathways of apoptosis may exist16. Studies have shown that one major difference between immunogenic and nonimmunogenic apoptosis is the expression of calreticulin (CRT) on the cell surface16. While normally expressed only in the endoplasmic reticulum, CRT undergoes caspase-1-dependent translocation to the cell surface, representing one of the earliest markers of cellular stress preceding cell death. Cell surface expression of CRT greatly enhances the uptake of dying tumor cells by dendritic cells (DC). Importantly, CRT is necessary, but not sufficient, for the immune response induced by apoptosis. The alarmin high mobility globulin box-1 (HMGB1) is released by cells dying in response to

immunogenic chemotherapy. HMGB1 (a non-histone chromatin-binding protein that regulates gene transcription and nuclear functions when present within the nucleus25) binds to the toll-like receptor 4 (TLR-4), triggering DC maturation and the activation of antigen-specific $CD4^+$ and $CD8^+$ T cell immunity12. Thus, CRT is essential for engulfment and subsequent DC maturation, whereas the HMGB1-TLR-4 interaction is required for optimal processing and presentation of tumor antigens from the dying tumor cells to T lymphocytes[12]. The clinical relevance of this pathway is revealed by a TLR-4 polymorphism leading to a single amino acid substitution (asp299gly) in the TLR-4 extracellular domain. This polymorphism reduces the binding of HMGB1 to human TLR-4 and inhibits HMGB1 mediated DC-T cell interactions. DCs from patients with a TLR-4 asp299gly mutation do not cross-present antigens from dying melanoma cells to $CD8^+$ T cells *in vitro*[12]. Moreoever, TLR-4 asp299gly mutation carriers, accounting for about 17% of a breast cancer cohort, had a higher risk of disease relapse after adjuvant treatment with anthracycline-based chemotherapy[12]. Similar findings have been reported for advanced colon cancer patients treated with oxaliplatin[24].

A second danger signal released by tumor cells dying in response to immunogenic chemotherapy is adenosine triphosphate (ATP)26,27. ATP binds to the P2RX7 purinergic receptor on DC, thereby stimulating assembly of the NOD-like receptor family, pyrin-domain-containing protein-3 (NRLP3)-dependent caspase-1 activation complex—the NRLP3 inflammasome--thus resulting in the proteolytic maturation of caspase-1, and the cleavage of pro-interleukin-1β to release soluble interleukin-1β (IL-1β). IL-1β then promotes the priming of interferon-γ (IFN-γ)-producing, tumor-specific $CD8^+$ T cells. Validating the clinical relevance of this pathway is the observation that breast cancer patients with a loss of function allele of P2RX7 relapsed sooner after anthracycline-based chemotherapy than breast cancer patients with a normal P2RX7 allele26,[27]. Other danger signals include crystalline uric acid (UA), heat shock proteins (hsp) 70 and 90, and the NK cell ligand NKG2D[28].

Homeostatic Proliferation

While some evidence suggests that lymphopenia may be associated with worse clinical outcomes29, data also suggest that profound lymphopenia can create an environment for "re-booting" the immune system30,31. If profound lymphopenia is established therapeutically (for example, with chemotherapy), a period of enhanced T cell proliferation follows, driven by cytokines that include interleukin-7, interleukin-15, and interleukin-213. This proliferation is driven by the recognition of self antigens, presented in the context of MHC Class I molecules. Thus, the tolerance for altered self characteristic of human tumors can be disrupted by homeostatic proliferation. This can be further skewed toward effective tumor immunity by the adoptive transfer of lymphocytes or vaccination during homeostatic T cell proliferation, which directs the re-established T cell repertoire toward a desired antigenic specificity32. Vaccine-induced tumor immunity can be enhanced when tumor-bearing mice are vaccinated with granulocyte-macrophage colony-stimulating (GM-CSF)-secreting tumor vaccines during early engraftment after syngeneic or allogeneic T cell-depleted bone marrow transplantation33,34, and further enhanced by donor leukocyte infusion from vaccinated donor mice35. Furthermore, breast 4T1 tumor-bearing mice that undergo surgical resection of existing tumor, followed by nonmyeloablative allogeneic stem cell transplantation and donor leukocyte infusions plus vaccination with a GM-CSF-secreting tumor vaccine developed immune responses capable of lysing metastatic 4T1 breast tumors36. These concepts have been tested in clinical trials of adoptive T cell transfer alone or combined with immunization during immune reconstitution[37,38]. Significant numbers of tumor antigen-specific T lymphocytes were shown to be present in individuals that displayed tumor regression after adoptive cellular therapy. Optimizing host

conditioning, and characterizing the kinetics, longevity, and functional quality of tumor antigen-specific T cells after immune reconstitution is essential for the optimal application of this approach to cancer care.

Regulatory T Cells (Treg)

It is now clear that a distinct population of suppressive T cells plays a key role in maintaining peripheral immune tolerance. $CD4^+CD25^+$ regulatory T cells (Treg) represent 5–10% of the peripheral T lymphocyte pool in mice and humans, and exist both to downregulate the normal immune response and prevent autoimmunity[39]. They express CTLA-4, GITR, and FoxP3, and secrete IL-10 and TGF-β. Treg inhibit $CD8^+$ T cell responses in an interleukin-2 (IL-2)-dependent manner through either direct cell-cell contact or the immunosuppressive effects of IL-10 or TGF-β. Treg potently suppress antitumor immune responses and accumulate in the peripheral blood and tumor microenvironment of patients with cancers of the breast, pancreas, ovary, stomach, lung, and liver[40].

Multiple groups have shown in preclinical models that treatment with low dose cyclophosphamide mitigates the influence of $CD4^+CD25^+$ Treg, allowing tumor immunity to emerge[41–44]. Cyclophosphamide reverses tumor-induced immunologic skew, promoting $CD4^+$ T helper type 1-driven tumor immunity[45,46]. Administering a low dose of cyclophosphamide prior to vaccination can facilitate the recruitment of latent, high aviditiy $CD8^+$ T cells that mediate tumor rejection[41]. Pretreatment of mice bearing HPV $E7^+$ tumors with cisplatin can decrease Treg and enhance antigen-specific $CD8^+$ T cell activity in response to E7-targeted DNA vaccination[47]. The activity of Treg can also be diminished in mice by giving cyclophosphamide, paclitaxel, or temozolamide in metronomic fashion[48–50].

The immune-modulating impact of chemotherapy drugs has also been explored in cnacer patients. Metronomic cyclophosphamide depletes $CD4^+C25^+$ Treg and restores T and NK effector function in patients with late stage cancers[51]. Standard paclitaxel-based chemotherapy in non small cell lung cancer (NSCLC) patients selectively decreased Treg through fas-mediated apoptosis, and upregulated the T helper type 1 cytokines IFN-γ and IL-2, and CD44 in $CD4^+$ and $CD8^+$ effector T cells[52]. Standard dose fludarabine can also decrease the number and function of Treg in patients with B cell chronic lymphocytic leukemia[53]. In addition, treatment of patients with metastatic colorectal carcinoma with gemcitabine and FOLFOX4, followed by subcutaneous GM-CSF and IL-2, produces a significant reduction of Treg in about 65% of patients; this finding is associated with a 70% objective response rate to therapy[54,55].

Myeloid-Derived Suppressor Cells (MDSC)

Myeloid-derived suppressor cells (MDSC) are a diverse population of cells that consists of myeloid progenitor cells and immature myeloid cells[56]. Murine MDSC are $GR1^+CD11b^+$, whereas human MDSC are typically $CD14^-CD11b^+CD33^+HLADR^-$. These cells expand in both tumor-bearing mice and cancer patients (up to 10-fold), and markedly suppress T cell-mediated immune responses through increased nitric oxide (NO) and arginase (ARG) production[56].

Chemotherapy can also influence MDSC. Cyclophosphamide causes a spike a MDSC numbers in tumor-free mice[57], and MDSC numbers increase in breast cancer patients treated with dose dense doxorubicin-cyclophosphamide (AC)[58]. Conversely, standard dose gemcitabine can eliminate MDSC in mice, thereby enhancing the activity of $CD8^+$ T cells and NK cells[59]. Cisplatin given prior to vaccination can decrease levels of peripheral MDSC in tumor-bearing mice[47].

Dendritic Cells

Dendritic cells (DC) are specialized antigen presenting cells that both induce self tolerance and stimulate T cell activation[60]. Conventional (as opposed to plasmacytoid) DC includes tissue-derived migratory DC and lymphoid tissue-resident DC. Migratory DC translocate from peripheral tissues to locoregional lymph nodes and are restricted to these lymph nodes, whereas resident DC are replenished by bone marrow-derived precursors61. Resident DC can be $CD8^+$, $CD4^+$, or $CD4^-CD8^-$[60]. Migratory DC can transfer antigen to resident DC, although the relative contribution of these two subsets to immunologic homeostasis remains unclear[60,62].

A number of chemotherapy drugs given at low doses have been reported to augment dendritic cells function, including cyclophosphamide, vincristine, vinblastine, paclitaxel, methotrexate, mitomycin-C, doxorubicin, and 5-aza-2'-deoxycytidine[63,64]. Of these drugs, the impact of cyclophosphamide and paclitaxel on DC maturation and function has been best described. Low dose cyclophosphamide selectively decreases $CD8^+$ resident DC compared to migratory or plasmacytoid DC, resulting in enhanced antigen presentation, augmented cytokine secretion, and some inhibition of Treg; this effect is abrogated by the adoptive transfer of $CD8^+$ DC to cyclophosphamide-treated mice, confirming the cell type modulated[65]. Extending this observation, nonmyeloablative doses of cyclophosphamide induce a rebound myelopoiesis with ensuing trafficking of DC to tumors, where they secrete more IL-12 and less IL-10 to favorably expand effector T cells over Treg[66]. Treatment with low dose cyclophosphamide has been associated with increased expression of DC maturation markers[67], and also regulates the production of type 1 interferons, thereby promoting the evolution of the $CD44^{hi}$ memory T cell response[68]. Highlighting the dose-dependent immunomodulatory activity of cyclophosphamide, lymphodepleting doses of cyclophosphamide induce the proliferation of DC in the bone marrow, followed by their expansion in the periphery[69]. These dendritic cells are capable of antigen presentation to T cells *in vivo*[70]. Notably, although lymphodepleting doses of cyclophopshamide also induce the systemic expansion of MDSC, these cells neither differentiate into DC nor impact their expansion or function[57].

The taxanes also have immune-modulating effects on DC. Paclitaxel has lipopolysaccharide (LPS)-mimetic activity, interacting through the murine toll-like receptor 4 (TLR-4) to facilitate DC activation and the secretion of pro-inflammatory cytokines[71,72]. A similar Myd88-dependent but TLR-4 independent effect suggests that an alternative TLR is responsible for the paclitaxel effect in humans73. Several preclinical studies have shown that paclitaxel can augment the activity of tumor vaccines, augmenting antigen-specific immunity and tumor-free survival[23,46,74,75]; this has been demonstrated for docetaxel as well[76,77]. Subclinical doses of paclitaxel given one day prior to (but not one week after) a HER-2-targeted GM-CSF-secreting cell-based vaccine augment $CD4^+$ T cell-dependent immunity in tolerant *neu*-N mice, delaying tumor outgrowth[46]. Interestingly, combining low dose paclitaxel with low dose cyclophosphamide synergize to delay tumor outgrowth in this preclinical model[78]. Paclitaxel-exposed DC precursors typically display higher levels of costimulatory molecules, maturation markers, IL-12 production, and augmented $CD8^+$ T cell priming and cytotoxic activity *in vitro* and *in vivo*[78,79].

Chemomodulation of Tumor Cell Immunogenicity

In addition to inducing apoptosis and immunologic cell death, some chemotherapy drugs render tumor cells more immunogenic in other ways. Chemotherapy can modulate the expression of both tumor antigens and molecules that regulate antigen processing and presentation. 5-fluorouracil can enhance the expression of carcinoembryonic antigen (CEA) in colon and breast carcinoma cells80, and 5'-aza-2'deoxycytidine81–84 can induce the

expression of a variety of cancer testis antigens and/or upregulate cell surface MHC Class I expression in distinct tumor cell lines (renal cell carcinoma, ovarian carcinoma, glioma, and melanoma), rendering them more susceptible to antigen-specific $CD8^+$ T cell-mediated lysis. Melphalan and mitomycin-C can upregulate expression of costimulatory B7 molecules, thus enabling tumor cells themselves to present antigen with a concurrent costimulatory signal[85,86]. Cytosine arabinoside (ara-C) increases the expression of B7-1 and B7-2 and decreases the expression of B7-H1, thus enhancing $CD8^+$ T cell-mediated killing in murine models of acute myelogenous leukemia (AML)[87]. This effect may underlie the ability of ara-C combined with a GM-CSF-secreting tumor cell vaccine to prolong the survival of AML-bearing mice88. Supporting the relevance of these observations for humans, a similar impact of ara-C on the expression of costimulatory molecules was observed for a majority of primary cultured human AML cells *in vitro*[87]. Multiple chemotherapeutic drugs can sensitize tumor cells to CTL-mediated apoptosis through fas- or perforin-granzyme-mediated pathways *in vitro*[89]. Furthermore, a single dose of cyclophosphamide can cure mice of malignant mesothelioma by sensitizing the mesothlioma cells to TRAIL-dependent $CD8^+$ T cell-mediated apoptosis even in the absence of effective antigen-specific T cell expansion[90].

More recently, the treatment of mice bearing Lewis lung carcinoma cells with low dose paclitaxel combined with an intratumoral DC vaccine was associated with enhanced numbers of tumor infiltrating $CD4^+$ and $CD8^+$ T cells, and elevated tumor-specific IFN-γ production by draining lymph node cells[91]. *In vitro*, paclitaxel pretreated tumor cells did not inhibit DC maturation to the extent that untreated tumor cells did. Furthermore, low dose paclitaxel altered the cytokine profile within the tumor site *in vivo*, with increased monocyte chemotactic protein-1 (MCP-1) and chemokine (C-X-C motif) ligand-10 (IP-10) levels and decreased levels of interleukin-1α (IL-1α). Finally, paclitaxel, cisplatin, and doxorubicin can sensitize tumor cells to T cell-mediated lysis by making them permeable to granzyme B[92]. This effect is perforin-independent, and mediated by the upregulation of mannose-6-phosphate in tumor cells. It was associated also with bystander lysis of tumor cells that did not express the tumor antigen target.

Clinical Trials of Chemoimmunotherapy

Clinical evidence suggests that the human immune system can be impacted by standard dose chemotherapy in both negative and positive ways (Table 2). Studies have shown that vaccination in close proximity to standard dose cytotoxic chemotherapy can inhibit vaccine-induced immune responses, facilitate levels of immune priming similar to those in the absence of chemotherapy, or increase the response to subsequent chemotherapy. One study of examined CEA-specific immune responses after 60 patients with advanced, CEA-expressing colorectal cancer were vaccinated with the canary pox vaccine ALVAC-CEA-B7.193. The number of CEA-specific T cells was decreased in patients who received a greater number of prior chemotherapy regimens and in those who had most recently received standard dose chemotherapy. Another study vaccinated patients with Stage II and III pancreatic carcinoma with a GM-CSF-secreting cell-based vaccine once after primary pancreaticoduodenectomy, and documented the induction of mesothelin-specific T cells post-vaccination[94,95]. Patients were then treated with 6 months of 5-fluorouracil-based chemoradiation, after which the mesothelin response was undetectable. Vaccine-induced mesothelin-specific immunity was restored only after three additional vaccines were given after chemoradiation was complete, suggesting suppression of the vaccine-induced immune response by subsequent chemoradiation. Based on promising evidence of immunologic activity in Phase I and II clinical trial of vaccine alone[96], a recent Phase III clinical trial (VITAL-2) of a GM-CSF-secreting, cell-based prostate cancer vaccine enrolled 405 chemotherapy-naïve men with symptomatic, hormone-refractory prostate cancer[97]. Subjects

were randomized to receive vaccination with 5×10^8 cell prime followed by 3×10^8 cell boost every 2 weeks for 12 cycles plus docetaxel 75 mg/m^2 every 3 weeks for 10 cycles, followed by 3×10^8 cell boost every 4 weeks, or docetaxel 75 mg/m^2 every 3 weeks plus daily prednisone 10 mg daily for 10 cycles. Halabi predicted survival time was 13 months for both arms. In August 2008, the Independent Data Monitoring Committee (IDMC) noted an imbalance of deaths between the two arms, and recommended study termination. The imbalance then lessened from 20 to 9, with 85 and 75 deaths on the two arms. All deaths were due to disease progression and death from prostate cancer. Importantly, the combination of this vaccine and standard dose docetaxel had not been tested in early Phase I and II clinical trials. Thus, the failure of this Phase III trial may be due in part due to a failure to appreciate the impact of standard dose docetaxel therapy on vaccine-induced tumor immunity.

On the other hand, standard chemotherapy can interact with established vaccine-induced immune responses for clinical benefit. A platform of hematopoietic cell transplantation after high dose chemotherapy with or without radiation followed by either adoptive cellular therapy or vaccine therapy has been tested by multiple groups. One study of cellular therapy transferred T cells specific for minor histocompatibility antigens after allogeneic stem cell transplantation for recurrent leukemia in 7 patients[98]. The primary toxicity was pulmonary. Transferred T cells persisted for up to 21 days, and 5 of the 7 patients achieved complete but transient remissions post-therapy, thus demonstrating the feasibility of this approach. Another report tested adoptive cellular therapy with autologous tumor-infiltrating lymphocytes (TIL) and IL-2 in 50 patients with metastatic melanoma equally randomized to receive conditioning with cyclophosphamide plus fludarabine with either 2 or 12 Gray of total body radiation (TBI)[99]. Nonmyeloablative chemotherapy alone resulted in response rates of 49%, whereas the addition of 2 or 12 Gray of TBI gave response rates of 52% and 72% respectively. Lymphodepletion was associated with elevated serum levels of IL-7 and IL-15, and objective responses correlated with the telomere length of the transferred lymphocytes. The use of GM-CSF-secreting, cell-based autologous vaccines after autologous stem cell transplantation for AML was evaluated in two distinct trials, with both demonstrating evidence of safety and immunogenicity[100,101].

Other groups have tested the impact of standard dose chemotherapy sequenced prior to or after vaccine therapy for solid tumors. A recent report of 36 HLA-A2$^+$ disease-free, stage II-IV melanoma patients tested standard dose dacarbazine (DTIC) at 800 mg/m^2 given one day prior to vaccination with melan-A/MART-1 plus gp100 melanoma peptide vaccination compared to vaccination alone[102]. DTIC enhanced the numbers of peptide-specific CD8$^+$ effector T cells, and the generation and persistence of peptide-specific effector memory CD8$^+$ T cells induced by peptide vaccination (Table 2). In another study, twenty-nine patients with extensive stage small cell lung cancer (SCLC) were vaccinated with DC transduced with an adenoviral vector expressing wild-type 53 (DC-Adp53)[103]. Although almost 60% of patients developed p53-specific immune responses with vaccine alone, all but one developed progressive disease. Interestingly, of those that received subsequent salvage chemotherapy, those that did develop p53-specific immunity were much more likely to display an objective clinical response. This observation suggests that previous vaccination may sensitize patients to respond to subsequent chemotherapy, and additional trials are studying the impact of previous therapeutic cancer vaccination with the DC-Adp53 vaccine on the subsequent response to chemotherapy in a prospective fashion104. A clinical trial of 32 patients with glioblastoma vaccinated with an autologous DC vaccine also demonstrated the apparent sensitization of patients with a vaccine-induced immune response to subsequent chemotherapy, with improved time to progression spanning post-vaccination salvage chemotherapy but not improved time to progression spanning the time period post-vaccination alone in patients who developed immunity105. A recent preclinical study using

the adoptive transfer of nonspecifically activated $CD4^+$ T cells as a chemosensitizer before the administration of chemotherapy demonstrated a marked enhancement of the cytotoxic effect of chemotherapeutic drugs both *in vitro* and *in vivo*, providing some preclinical experimental validation for these clinical observations[106].

Work has also examined the impact of concurrent chemotherapy on cancer vaccines (Table 2). A study of prime-boost vaccination alone or with concurrent docetaxel therapy enrolled 28 patients with metastatic, hormone refractory prostate cancer107. The vaccination regimen consisted of a priming immunization with a recombinant vaccinia virus expressing prostate specific antigen (PSA) admixed with recombinant vaccinia virus expressing B7.1 followed by recombinant fowlpox virus expressing PSA. Vaccination resulted in over a 3-fold increase in PSA-specific T cells by ELISPOT at 3 months, regardless of concurrent docetaxel. Median progression-free survival for vaccinated patients on docetaxel was 6.1 months compared to 3.7 months for a historical cohort of patients treated with docetaxel alone. A prospective study to follow up on observations that prior chemotherapy may inhibit cancer vaccine activity randomized 118 patients with metastatic colorectal cancer to receive ALVAC alone for 3 vaccination cycles followed by ALVAC given with chemotherapy (fluorouracil/leucovorin/irinotecan), ALVAC with tetanus toxoid adjuvant for three vaccination cycles followed by ALVAC given with chemotherapy, or 4 cycles of chemotherapy followed by 4 cycles of ALVAC vaccination in patients without disease progression[108]. Increases in CEA-specific T cells were detected in 50%, 37%, and 30% of patients respectively, suggesting that systemic chemotherapy did not impact vaccine-induced immune responses. Another study tested the combination of three cycles of standard irinotecan/high dose 5-fluorouracil/leucovorin and concurrent vaccination with a carcinoembryonic antigen (CEA)-derived peptide, followed by vaccination with peptide alone in patients with newly diagnosed metastatic colorectal cancer109. Almost half of treated patients developed CEA-specific T cell responses by intracellular cytokine staining.

Several clinical trials have now been reported that use low, immune-modulating doses of chemotherapy specifically to enhance the activity of cancer vaccines rather than to lyse tumor cells directly (Table 2). A number of Phase II studies designed to mitigate the influence of suppressor T cell as they were defined some 35 years ago first showed that patients receiving cyclophosphamide at 300 mg/m^2 three days before vaccination with a clustered carbohydrate antigen (STn)-keyhole limpet hemocyanin (KLH) vaccine developed higher antibody titers and enjoyed longer survival[110]. In follow up to these early studies, a Phase III clinical trial of 1,028 women with metastataic breast cancer randomized 505 women to receive cyclophosphamide plus KLH and 523 women to receive cyclophosphamide plus STn-KLH111. In this trial, no difference in time to disease progression or overall survival emerged. This approach has also been tested in combination with GM-CSF-secreting tumor vaccines for a variety of cancer. One trial enrolled patients with metastatic pancreatic cancer, testing immune-modulating doses of cyclophosphamide (300 mg/m^2) given one day prior to immunization with a GM-CSF-secreting, cell-based pancreas cancer vaccine[112]. This study revealed a trend toward increased vaccine-activated $CD8^+$ T cell immunity specific for mesothelin, and a corresponding trend toward increased clinical benefit with cyclophosphamide-modulated vaccination compared to vaccination alone. Similar findings regarding clinical benefit were reported in small trial of the same vaccine platform in patients with NSCLC treated with immune-modulating doses of cyclophosphamide at 300 mg/m^2 given the day prior to vaccination; a transient decrement in Treg numbers with time after cyclophosphamide treatment was also observed [113]. Another clinical trial with an innovative factorial response surface design tested a HER-2-positive, GM-CSF-secreting cell-based breast tumor vaccine alone or with a range of low doses of cyclophosphamide (0, 200, 250, 350 mg/m^2) given one day prior to vaccination and doxorubicin (0, 15, 25, 35 mg/m^2) given seven days after vaccination in patients with stable

metastatic breast cancer[114]. The vaccine alone induced *de novo* HER-2-specific delayed type hypersensitivity (DTH), with low levels of HER-2-specific antibody also induced. The addition of cyclophosphamide at 200 mg/m^2 maintained the DTH response and further augmented HER-2-specific antibody levels, but cyclophosphamide doses of 250 mg/m^2 or higher abrogated vaccine-induced immunity. The chemotherapy dose combination that optimized vaccine activity was 200 mg/m^2 cyclophosphamide, and 35 mg/m^2 doxorubicin. It is notable that most clinical cancer vaccine trials have historically used cyclophosphamide at doses of 250–300 mg/m^2 for immune-modulation to enhance vaccine activity, suggesting a narrow therapeutic window for the immunomodulatory activity of low dose cyclophosphamide.

Conclusions

We have made a great deal of progress in understanding the cellular and molecular basis of both the fundamental immunobiology of the host-tumor interaction, and the impact of cancer chemotherapy drugs on that interaction. Armed with this knowledge, the potential for maximizing the bioactivity and clinical benefit of immune-based therapies has never been greater. Strategically integrating immunotherapies with chemotherapy drugs in order to shape the overall host milieu and the local tumor microenvironment and ameliorate distinct mechanisms of immune tolerance and suppression will ultimately support a vigorous and sustained antitumor immune response. Carefully dissecting the proper dose and timing of drugs for integrating with immune-based therapies in clinically relevant laboratory models and early phase clinical trials will accelerate late stage clinical development, making clinically meaningful chemoimmunotherapy for cancer care a reality.

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Table 1

The Immunomodulatory Effects of Chemotherapy Drugs

Chemotherapy Drug	Immunologic Process	Mechanism
Doxorubicin Daunorubicicn Mitoxantrone	Antigen uptake	Cell surface calreticulin
Doxorubicin Daunorubicicn Mitoxantrone Oxaliplatin	Antigen processing	HMGB1 release
High dose chemotherapy	Homeostatic proliferation	Cytokines (IL-7, IL-15, IL-21); inhibition of Treg
Cyclophosphamide Cisplatin Paclitaxel Temozolomide Fludaribine Gemcitabine/FOLFOX4	Regulatory cell function	Inhibition of $CD4^+CD25^+$ $FoxP3^+$ regulatory T cells
Gemcitabine 5-fluorouracil	Regulatory cell function	Reduced MDSC numbers
Cyclophosphamide Cyclophosphamide/Doxorubicin	Regulatory cell function	Increased MDSC numbers
Cyclophosphamide Paclitaxel Bleomycin Melphalan	Immunologic skew	Promotes T helper type 1 immunity
Cyclophosphamide Vincristine Vinblastine Paclitaxel Methotrexate Mitomycin-C Doxorubicin 5-aza-2'-deoxycytidine	Antigen presentation	Augment DC function Cyclophosphamide: decreased $CD8^+$ DC Paclitaxel: through TLR-4/MyD88
5-aza-2'-deoxycytidine	Antigen processing	Increasing MHC Class I expression
5-aza-2'-deoxycytidine 5-Fluorouracil	Antigen processing	Increasing tumor antigen expression
Melphalan Mitomycin-C Cytosine arabinoside	Co-stimulation	Upregulate B7-1/B7-2 expression
Cytosine arabinoside	Counter-stimulation	Downregulate B7-H1 expression
Cyclophosphamide Paclitaxel Cisplatin Doxorubicin	Target cell lysis	Upregulation of lytic sensitivity to TRAIL, fas, granzyme-B

Abbreviations: HMGB-1=high mobility box globulin-1; IL=interleukin; MDSC=myeloid-derived suppressor cells; DC=dendritic cells; TLR=toll-like receptor; MHC=major histocompatibility complex

Table 2

Clinical Trials of Combinatorial Chemoimmunotherapy

Standard Dose Chemotherapy				
Patient Population	**n**	**Vaccine**	**Drug**	**Immunologic Outcome**
metastatic colon cancer	17	CEA peptide	standard 5-fluorouracil, leucovorin, irinotecan	CEA-specific immunity in 50% of vaccinated patients
metastatic colon cancer	118	ALVAC-CEAB7.1	standard 5-fluorouracil, leucovorin, irinotecan	no inhibition of CEA-specific immunity
early stage pancreas cancer	60	GM-CSF-secreting mesothelin^{+} pancreas tumor cells	vaccine once, then 5-fluorouracil-based chemo-radiation, then 3 additional vaccines	Inhibiton of vaccine-induced mesothelin T cell responses by subsequent chemoradiation, restored by 3 additional vaccines
metastatic prostate cancer	28	PSA vaccinia/fowl pox	standard docetaxel and dexamethasone	no inhibition of PSA-specific immunity
metastatic melanoma	10	Melan-A/gp100 peptide	DTIC 0 mg/m^2 (n=5) vs. DTIC 800 mg/m^2 (n=5)	increased peptide-specific immunity with DTIC 800 mg/m^2
metastatic hormone-refractory prostate cancer	405	GM-CSF-secreting prostate tumor cells	Vaccine plus docetaxel vs. docetaxel plus prednisone	NR, study terminated
Low Dose Immunomodulatory Chemotherapy				
Patient Population	**n**	**Vaccine**	**Drug**	**Immunologic Outcome**
metastatic breast cancer	1028	KLH (n=505) KLH-STn (n=523)	CY 300 mg/m^2	NR, no clinical difference
metastatic pancreas cancer	50	GM-CSF-secreting mesothelin^{+} pancreas tumor cells	CY 0 mg/m^2 (n=30) CY 300 mg/m^2 (n=20)	increased mesothelin-specific immunity with CY 300 mg/m^2
metastatic breast cancer	28	GM-CSF-secreting HER-2^{+} breast tumor cells	CY 0, 200, 250, 350 mg/m^2 DOX 0, 15, 25, 35 mg/m^2 (factorial design of various dose combinations)	increased HER-2-specific immunity with CY 200 mg/m^2 plus DOX 35 mg/m^2

Abbreviations: CEA=carcinoembryonic antigen; PSA=prostate-specific antigen; DTIC=dacarbazine; KLH=keyhole limpet hemocyanin; STn=clustered carbohydrate antigens; CY=cyclophosphamide; NR=not reported; GM-CSF=granulocyte-macrophage colony-stimulating factor; DOX=doxorubicin