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Chemoimmunotherapy: reengineering tumor immunity

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Abstract

Cancer chemotherapy drugs have long been considered immune suppressive. However, more recent data indicate that some cytotoxic drugs effectively treat cancer in part by facilitating an immune response to the tumor when given at the standard dose and schedule. These drugs induce a form of tumor cell death that is immunologically active, thereby inducing an adaptive immune response specific for the tumor. In addition, cancer chemotherapy drugs can promote tumor immunity through ancillary and largely unappreciated immunologic effects on both the malignant and normal host cells present within the tumor microenvironment. These more subtle immunomodulatory effects are dependent on the drug itself, its dose, and its schedule in relation to an immune-based intervention. The recent approvals of two new immune-based therapies for prostate cancer and melanoma herald a new era in cancer treatment and have led to heightened interest in immunotherapy as a valid approach to cancer treatment. A detailed understanding of the cellular and molecular basis of interactions between chemotherapy drugs and the immune system is essential for devising the optimal strategy for integrating new immune-based therapies into the standard of care for various cancers, resulting in the greatest long-term clinical benefit for cancer patients.

Keywords

Chemotherapy; Cyclophosphamide; Vaccine; Immunotherapy; Chemoimmunotherapy; Clinical trials

Introduction

Cancer therapy is designed to specifically integrate distinct treatment modalities in the most effective way to achieve the highest cure rate. Surgery and radiation therapies are used for locoregional disease control, whereas systemic therapies are used to treat micrometastatic or widespread metastatic cancers and hematologic malignancies. While systemic therapies have historically been given after local measures have been undertaken to remove the primary tumor, they are increasingly used prior to definitive local treatment both to achieve systemic disease control earlier and to evaluate the responsiveness of the tumor to treatment. Regardless of the timing in relation to local therapy, these systemic treatments—

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chemotherapy, endocrine therapy, small molecular targeted therapies and monoclonal antibodies—are designed to decrease the likelihood of relapse due to micrometastatic disease.

Therapies that induce an immune response to tumors—immunotherapies—have been investigated for over 100 years as attractive strategies for cancer treatment [1]. While initially tested alone, newer work demonstrates that immunotherapy can complement standard cancer treatments. With the FDA approval of two immunotherapies for clinical use, cancer immunotherapy has taken its place in the clinic as a *bona fide* approach to cancer treatment. The first cancer vaccine (sipuleucel-T, Provenge) was approved for advanced prostate cancer [2], and the first immune checkpoint inhibitor targeting the negative regulatory molecule cytotoxic T lymphocyte antigen-4 (CTLA-4) on T cells (ipilimumab, Yervoy) was approved for the treatment of advanced melanoma [3]. More recently, two clinical trials of antibodies that target the negative immune checkpoint molecule PD-1 on T cells and its ligand B7-H1/PD-L1 on tumor cells were unexpectedly successful, with durable response rates of 20–25 % in advanced melanoma, renal cell cancer, and nonsmall cell lung cancer (NSCLC) [4, 5]. Despite the promise of these immunologically targeted approaches to cancer treatment, a number of developmental challenges remain to be solved if we are going to realize the full potential of cancer immunotherapy. First, understanding the differences in immunobiology between the distinct histologies and biologic subtypes of cancer will be critical for identifying the optimal antigen and/or immunologic pathway to target for a particular cancer. Second, dissecting mechanisms of intrinsic and adaptive therapeutic resistance to immune-based treatments will be critical for ensuring clinical success. Finally, delineating the impact of established cancer drugs and standard cancer treatment modalities on the immune system and on tumor immunobiology itself will be critical for the most effective integration of immune-based cancer therapy into state-of-the-art multimodality cancer care. Current data suggest that combining chemotherapy in standard and novel ways with immune-based interventions will have great potential for optimizing the clinical outcomes of cancer patients.

Challenges to effective natural and therapeutic cancer immunity

An effective immune response to cancers should result in the regression of established tumors and should also be able to prevent the development of a new cancer. However, multiple factors present a barrier to the antitumor immune response. Because tumors are frequently perceived by the immune system as “self,” the mechanisms that control the development of autoreactive immune responses (and thus, autoimmune disease) also serve to preclude the development of an effective immune response to cancer (reviewed in [6]). The deletion of high-avidity autoreactive T cells during thymic education leaves in place a pool of low-avidity T cells specific for self-antigens (including tumor antigens) that are functionally suboptimal. Regulatory T cells provide a backup mechanism of regulation that helps to keep these cells and any high-avidity T cells that escape deletion from attacking both normal tissues and tumors. Furthermore, crosstalk between progressing tumors and the host immune system results in multiple superimposed mechanisms of additional regulation and immune escape that serve to keep the immune response to tumors shut down. A variety of immune cells that promote tumor growth and inhibit tumor-associated immune responses, or both, accumulate within the tumor and its locoregional draining lymph nodes. In particular, these include CD4⁺CD25⁺FOXP3⁺ regulatory T cells (Tregs), CD4⁺interleukin-17-producing T helper cells, myeloid-derived suppressor cells (MDSCs), and tumor-associated macrophages (TAMs). Additional features of the tumor microenvironment further silence the antitumor immune response, including high levels of suppressive intratumoral cytokines [transforming growth factor- β (TGF- β), tumor necrosis factor (TNF), and interleukin-10 (IL-10)], the constitutive or induced expression of immune checkpoint molecules by the tumor cells (PD-L1, B7-H4), and various other phenotypic

alterations that lead to immune escape (the loss of tumor antigens and other molecules essential for antigen processing and presentation). In addition, many standard and high-dose chemotherapy regimens can be immunosuppressive, by either frankly inducing lymphopenia or contributing to lymphocyte dysfunction. Other medications used as a critical part of anticancer therapies, or given as adjunctive medications to mitigate the side effects of therapy, can also adversely affect the immune system.

Because surgery, radiotherapy, chemotherapy, and targeted therapies are widely used to treat most established cancers, the optimal integration of immune-based therapies with these standard modalities to minimize antagonistic interactions between different treatment modalities is of great importance. In addition, multiple lines of evidence suggest that chemotherapy in particular may have the potential to augment cancer immunity in several distinct ways. From a simple point of view, giving immune-based therapy in the setting of minimal residual disease—after optimal tumor debulking by surgery and/or chemotherapy—simply mitigates the negative impact of tumor burden on the potency and efficacy of the antitumor immune response. Some forms of chemotherapy-induced cell death can be highly immunogenic, and chemotherapy-induced apoptosis in particular can augment cross-priming of the tumor-specific T cell response [7]. For example, standard neoadjuvant paclitaxel therapy for early breast cancer can result in the development of new intratumoral immune cell infiltrates, with the clinical and pathologic response directly correlating with the tumor cell apoptotic response [8]. Chemotherapy may promote antitumor immunity in more subtle ways, modulating the expression of tumor antigens and immune checkpoint molecules as well as molecules more directly involved in antigen processing and presentation [reviewed in 9]. Finally, chemotherapy may impinge on mechanisms of immune tolerance and suppression to promote antigen-specific immunity [9]. A seminal study demonstrated that a single low dose of cyclophosphamide given 1–3 days before antigen exposure overcomes systemic immune tolerance to enhance both the antibody and the T cell response [9]. Conversely, concurrent or subsequent administration induced antigen-specific tolerance. It is clear that carefully choosing the chemotherapy drugs, their dose, and their timing of administration in relation to immune-based therapy is essential for engaging the potential additive and synergistic clinical activities of chemotherapy and immunotherapy, and leading to maximal clinical benefit.

Mechanisms of chemotherapy-induced immunomodulation

Chemotherapy can promote tumor immunity in two major ways: (a) through its intended therapeutic effect of killing cancer cells by immunogenic cell death, and (b) through ancillary and largely unappreciated effects on both the malignant and normal host cells present within the tumor microenvironment. A growing body of data demonstrates that some chemotherapy drugs given at their standard dose and schedule exert their antitumor effect at least in part by inducing immunogenic cell death [10]. This process can involve either the pattern recognition receptor toll-like receptor-4 (TLR-4) or the purinergic receptor P2RX7. The clinical relevance of these immunogenic cell death pathways is suggested by the association of loss of function polymorphisms of both TLR-4 and P2RX7 with a higher risk of breast cancer relapse after adjuvant anthracycline-based chemotherapy [11, 12]. Consistent with these observations, advanced stage colon cancer patients treated with oxaliplatin chemotherapy have a shorter progression-free survival (PFS) and overall survival (OS) if they harbor the TLR-4 loss of function polymorphism than if they carry the normal TLR-4 allele [13]. Notably, these same loss of function alleles seem to have no effect on clinical outcome in patients with NSCLC, suggesting that the potential for immunogenic cell death is either dependent on the tumor biology, the chemotherapy, or both [14]. In addition to immunogenic tumor cell death, there are a number of ancillary mechanisms by which chemotherapy may promote the antitumor immune response [9]. These include enhanced

intrinsic tumor cell immunogenicity, the modulation of a variety of immunoregulatory cells [including Tregs and other CD4⁺ T cells, MDSCs, and dendritic cells (DCs)], and the induction of homeostatic proliferation.

Enhanced tumor cell immunogenicity

Various chemotherapy agents can modulate intrinsic tumor cell immunogenicity in multiple ways (summarized in Table 1). Chemotherapeutics can upregulate the expression of tumor antigens (CEA and cancer–testis antigens, for example) as well as MHC class I molecules, thereby increasing capacity for tumor antigen presentation. Some drugs induce the expression of co-stimulatory molecules such as B7-1 on the surface of tumor cells, rendering them better able to present tumor antigens to achieve effective immune activation. Other drugs decrease the expression of immune checkpoint molecules such as B7-H1/PD-L1 and B7-H4 on the tumor cell surface, thereby preventing infiltrating T cells from being shut down. Finally, other chemotherapy drugs can sensitize tumor cells to CD8⁺ T cell-mediated lysis through fas-, perforin-, and granzyme-B-dependent mechanisms.

Regulatory T cells and other CD4⁺ T cells

CD4⁺ T cells can be classified into several distinct subsets, including Tregs, CD4⁺ T helper type 1 cells, CD4⁺ T helper type 2 cells, and CD4⁺ T helper type 17 cells [28]. The fact that CD4⁺ T cells are characterized by considerable phenotypic and functional plasticity complicates their classification, but it is clear that some chemotherapy drugs can promote or suppress functions attributed to each of these subsets.

Tregs represent 5–10 % of the peripheral CD4⁺ T lymphocyte pool and play a key role in maintaining peripheral tolerance and preventing autoimmunity by inhibiting CD8⁺ T cell responses in an interleukin-2-dependent manner [29]. These cells expand in the peripheral blood and within the tumors of patients with breast, pancreas, ovary, stomach, lung, and liver cancers [30]. There, they potently suppress the antitumor immune response. Many groups have shown in both preclinical models and in cancer patients that several chemotherapy drugs can abrogate the suppressive influence of Tregs and allow effective antitumor immunity to emerge (Table 2). Both cyclophosphamide and cisplatin can decrease Tregs and enhance antigen-specific CD8⁺ T cell activity in murine models [31–37]. Also in murine models, the metronomic administration of cyclophosphamide, paclitaxel, or temozolomide can decrease Treg activity [38–40]. Clinically, cyclophosphamide at doses of 200–300 mg/m² given 1 day prior to vaccination and 600 mg/m² given 7 days prior to vaccination has been shown to decrease Tregs [30]. In addition, metronomic cyclophosphamide alone can deplete Tregs and restore T and NK cell effector function in advanced cancer patients [41]. Together, these findings illustrate the importance of dose, schedule, and therapeutic intent in determining the best administration schema for cyclophosphamide. In addition, fludarabine- or paclitaxel-based chemotherapy can decrease Tregs in cancer patients [42–44], an effect sometime associated with improved clinical response [45]. In addition, several chemotherapy drugs can shift the CD4⁺ T helper cell phenotype from type 2 to type 1, thereby reversing immunologic skew and promoting tumor immunity [9, 36]. Finally, cyclophosphamide can also promote the CD4⁺ T helper type 17 cell phenotype and function [46, 47].

Myeloid-derived suppressor cells (MDSCs)

MDSCs are a heterogeneous population of myeloid cells that progressively accumulate in cancer patients and potently inhibit T cell-mediated immune responses through the production of nitric oxide and arginase [48]. In murine models, cyclophosphamide administration increases peripheral MDSC numbers in tumor-free mice [49]. In patients, MDSC numbers increase in breast cancer patients treated with standard doxorubicin and

cyclophosphamide (AC) chemotherapy [50]. Standard doses of gemcitabine, 5-fluorouracil, and cisplatin can decrease peripheral MDSC in mice, thereby enhancing the potential for expanding CD8⁺ T cells in vitro and in vivo, both naturally and after vaccination [37, 51, 52]. Docetaxel decreases MDSC and increases CD8⁺ T cell activity in a murine model of breast cancer [53]. Specifically, it selectively decreases regulatory MDSC while sparing other MDSC and polarizing them toward a functional antitumor phenotype. These findings together suggest distinct chemotherapy drugs impact MDSCs in idiosyncratic ways.

Dendritic cells (DCs)

Multiple chemotherapy drugs given at low doses can enhance the function of DCs; these include cyclophosphamide, vincristine, vinblastine, paclitaxel, docetaxel, methotrexate, mitomycin-C, doxorubicin, and 5-aza-2'-deoxycytidine [54, 55]. Low-dose cyclophosphamide differentially decreases the bone marrow-derived CD8⁺ tissue-resident DC relative to migratory or plasmacytoid DC, thereby increasing effective antigen presentation and cytokine secretion [56]. It also promotes DC maturation [57] and induces the production of type 1 interferons [58], ultimately resulting in the evolution of a durable CD44^{hi} memory T cell response. Nonmyeloablative doses of cyclophosphamide induce compensatory myelopoiesis, which promotes the trafficking of DC to tumors [59]. There, they secrete high levels of IL-12 relative to IL-10, favoring the function of effector T cells relative to Tregs. Lymphodepleting doses of cyclophosphamide cause the proliferation of DC within the bone marrow, followed by their expansion in the periphery [60]. These DCs are capable of effectively presenting antigens to T cells [61].

The taxanes also impact DC phenotype and function. Low-dose paclitaxel facilitates DC maturation and proinflammatory cytokine secretion (particularly IL-12) in a TLR-dependent fashion [62–64]. This results in augmented CD8⁺ T cell priming and cytotoxic activity [65, 66]. In vivo, subclinical doses of paclitaxel given 1 day prior to a human epidermal growth factor receptor-2 (HER-2)-targeted granulocyte and macrophage colony-stimulating factor (GM-CSF)-secreting tumor vaccine shifts the CD4⁺ T helper response from type 2 to type 1 in mice immunologically tolerant to HER-2, delaying tumor outgrowth [36]. Combining paclitaxel and cyclophosphamide at low doses synergistically delays the outgrowth of HER-2⁺ tumors in this model [65]. Both paclitaxel and docetaxel at low doses can augment the activity of other vaccine platforms in other preclinical models, enhancing tumor-specific immune responses and tumor-free survival [67–70].

Homeostatic proliferation

A growing body of evidence suggests that the simple presence of lymphopenia may be associated with worse clinical outcomes [71]. Conversely, lymphopenia induced with therapeutic intent can create space for reshaping the repertoire of immune cells available for fighting cancer, with the potential for improving clinical outcomes [72, 73]. Strategically inducing significant lymphopenia creates both space and a cytokine milieu that includes high levels of IL-7, IL-15, and IL-21, promoting the proliferation of T cells that recognize self [74]. This proliferating T cell repertoire can be further skewed toward a desired antigenic specificity by vaccination or by the adoptive transfer of antigen-specific lymphocytes during homeostatic proliferation [75]. In a preclinical lung cancer model, the chemotherapy doublet cisplatin/vinorelbine induces a lymphopenia that promotes the vaccine-induced expansion of effector T cells relative to Tregs through the influence of IL-7 [76]. Vaccine-induced tumor immunity can also be augmented when tumor-bearing mice are vaccinated during early engraftment after either syngeneic or allogeneic T cell-depleted bone marrow transplantation; it is further enhanced by the infusion of donor leukocytes from vaccinated donor mice [77–79]. These strategies have been tested in clinical trials of adoptive T cell transfer alone or combined with vaccination during immune reconstitution

with some success [80–82]. In contrast, after induction timed sequential therapy for newly diagnosed acute myelogenous leukemia (AML), recovering T lymphocytes were predominantly peripherally derived, phenotypically activated, and functionally suppressive Tregs, where the expansion was antigen-driven [83]. It is clear that optimizing treatment regimens that induce lymphopenia, and characterizing the kinetics and longevity of T cell recovery as well as the phenotype and functional characteristics of lymphocyte recovery, will be essential to harness the power of homeostatic proliferation for targeted tumor therapy.

Clinical trials of chemoimmunotherapy

Clinical trials incorporating cancer vaccines, immune checkpoint blockade, or adoptive cellular therapy have typically tested the immunotherapies integrated with standard-dose chemotherapy, sequenced with low-dose chemotherapy specifically designed for immune modulation, or given in the setting of high-dose chemotherapy designed to reboot the immune system through cytokine release and homeostatic proliferation. These clinical trials are summarized in Tables 3 and 4.

Standard-dose chemotherapy and immunotherapy

Many standard-dose chemotherapy regimens suppress the antitumor immune response by directly decreasing lymphocyte numbers or inhibiting lymphocyte functions. Glucocorticoids are a critical component of many chemo-therapy regimens, compounding the immune suppressive effects of cytotoxic therapies through their direct lymphocytic effects. However, certain chemotherapy agents can generate tumor-specific immune responses as part of their mechanism of action, primarily by inducing immunogenic cell death. This duality underlies the observation that standard-dose chemotherapy can repress vaccine-induced immune responses, augment vaccine-induced immune responses, or have no impact on vaccine activity. In part, the impact of chemotherapy on vaccine-induced tumor immunity depends on its timing of administration in relation to vaccination.

Concurrent standard-dose chemotherapy may inhibit immunotherapy—One study examined immune responses to vaccination with the canary pox vaccine ALVAC-CEA-B7.1 in patients with advanced carcinoembryonic antigen (CEA)-expressing malignancies [84]. A retrospective analysis showed that a greater number of prior chemotherapy regimens and more recent standard-dose cytotoxic therapy were both associated with lower numbers of vaccine-induced CEA-specific T cells. A phase II study integrated a mesothelin-expressing, GM-CSF-secreting pancreas tumor vaccine with standard therapy for stage II and III pancreas cancers [85]. Participants developed mesothelin-specific T cell responses after one vaccination given prior to pancreaticoduodenectomy and then went on to receive 6 months of 5-fluorouracil-based chemoradiation, with ensuing loss of the mesothelin-specific T cell response. Three additional vaccinations given after adjuvant chemoradiation restored the mesothelin-specific T cell response, suggesting that surgery and chemoradiation had suppressed vaccine-induced immunity. A phase III study tested a GM-CSF-secreting prostate tumor vaccine with standard-dose docetaxel chemotherapy and randomized patients to vaccination with ten cycles of docetaxel every 3 weeks without prednisone or ten cycles of docetaxel every 3 weeks with prednisone 10 mg daily [86, 87]. Due to an imbalance of deaths (all due to disease progression and death from prostate cancer) on the experimental arm, the study was terminated prior to completion. With subsequent follow-up, the imbalance of deaths lessened from 20 to 9. Two major factors likely contributed to the failure of this trial. First, the standard chemotherapy regimen for advanced prostate cancer includes both docetaxel and prednisone, but the prednisone was not given to vaccinated patients due to concern for

immune suppression. Second, the vaccine itself had never been tested in the setting of chemotherapy before. This dose and schedule of docetaxel may have impaired vaccine activity.

Concurrent standard-dose chemotherapy may facilitate immunotherapy—In one study, a CEA-specific peptide vaccine was given first concurrently with 3 cycles of high dose 5-fluorouracil/leucovorin plus standard dose irinotecan and then alone in 17 patients with metastatic colorectal cancer at first relapse. There were 6 objective responses, and 5 patients with stable disease and 6 patients with progressive disease [88]. Immunologically, about 50 % of participants developed CEA-specific T cell responses, whereas during active chemotherapy, recall CD8⁺ T cell immunity to Epstein–Barr virus (EBV) and cytomegalovirus (CMV) declined by about 14 %. The impact of chemotherapy (5-fluorouracil, leucovorin, and irinotecan) on immunity induced by the ALVAC-CEA-B7.1 vaccine was further explored prospectively in 118 patients with metastatic colorectal cancer [89]. Three groups were treated with (a) vaccine alone for 3 cycles followed by vaccine and concurrent chemotherapy; (b) vaccine with tetanus toxoid adjuvant given for 3 cycles followed by vaccine and concurrent chemotherapy; or (c) 4 cycles of chemotherapy followed by 4 cycles of vaccination in patients without disease progression on chemotherapy. Although no significant differences in clinical or immunologic responses between the three groups emerged, CEA-specific T cells increased between 30 and 50 % across the groups, suggesting that chemotherapy did not impair vaccine-induced immunity. Another study tested a prime/boost recombinant vaccinia virus (rVV)/fowl pox (rF) vaccination regimen for patients with metastatic hormone-refractory prostate cancer [90]. Patients were vaccinated with rVV expressing prostate-specific antigen (PSA) admixed with rVV expressing the co-stimulatory molecule B7.1, followed by vaccination with rF-PSA alone or with concurrent weekly docetaxel (30 mg/m²). PSA-specific T cells increased by greater than threefold over 3 months regardless of concurrent docetaxel therapy, and median progression-free survival (PFS) was 6.1 months compared to 3.7 months for a historical patient cohort treated with weekly docetaxel. Another trial in 25 patients with metastatic melanoma showed that the telomerase-specific peptide vaccine GV1001 can be combined with temozolomide at 200 mg/m² orally for 5 days every 4 weeks, with the GV1001 vaccinations being given as 8 injections over 11 weeks [91]. The majority (78 %) of patients developed a GV1001-specific immune response characterized by high IFN- γ /IL-10 ratios, polyfunctional cytokine profiles, and the capacity to recognize naturally processed antigens. Furthermore, long-term immunologic memory was associated with clinical benefit. Another trial tested concurrent chemoradiation (2 Gray for 30 days and weekly docetaxel 20 mg/m²) and GV1001 vaccination in 23 patients with inoperable stage III NSCLC [92]. Here, GV1001-specific immunity was observed in 16 of 20 evaluable patients (80 %), with durable responses in 13 subjects. An immune response was associated with a median PFS of 371 days, compared with 182 days for immune nonresponders. Finally, a seminal paper demonstrated that concurrent therapy with dacarbazine and ipilimumab was associated with improved overall survival (OS) in patients with untreated metastatic melanoma [93].

Sequencing standard-dose chemotherapy before or after immune-based therapy may augment immunotherapy—Several studies have examined phased treatment with standard-dose chemotherapy and immunotherapies, where most studies explored giving chemotherapy first. A study of 36 patients with stage 2–4 melanoma and no evidence of disease were treated either with 800 mg/m² dacarbazine (DTIC) 1 day prior to vaccination with melan-A/MART-1/gp-100 peptides, or with the peptide vaccine alone [94]. The administration of DTIC prior to vaccination induced the expression of genes involved in the immune response, increased numbers of peptide-specific CD8⁺ T cells, and promoted the generation of a long-lived memory T cell response. Further analyses also revealed a

broader T cell receptor diversity, higher T cell avidity, greater tumor reactivity, and a trend toward longer survival in DTIC-pretreated patients [95]. Finally, a pilot clinical trial of 24 patients with metastatic cancers tested type 1 DCs loaded with autologous tumor lysates given as 2 cycles of 4 daily vaccinations sequenced with cyclophosphamide at 600 mg/m² 7 days prior to vaccination, followed by GM-CSF days 1–4 after vaccination and pegylated IFN- α days 1 and 8 after vaccination [96]. This study showed that cyclophosphamide pretreatment reduced Tregs to levels observed in healthy individuals. T cell responses specific for DC+ tumor lysate were observed in 4 of 11 tested patients, and 5 patients demonstrated disease stabilization.

Immune checkpoint blockade has also been tested with standard-dose chemotherapy (Table 4). A study in NSCLC and extensive-stage SCLC examined the use of concurrent standard chemotherapy with 4 cycles of carboplatin and paclitaxel plus the immune checkpoint inhibitor ipilimumab followed by 2 cycles of carboplatin and paclitaxel as compared to phased treatment with 2 cycles of standard carboplatin and paclitaxel followed by 4 cycles of ipilimumab plus carboplatin and paclitaxel [97, 98]. In both patient groups, improved immune-related PFS was observed for phased ipilimumab, where standard chemotherapy was given first for two cycles before ipilimumab was added. Finally, a study in 30 patients with metastatic breast cancer tested the immune checkpoint modulator IMP321 (recombinant soluble LAG3Ig fusion) every 2 weeks preceded by standard dose weekly paclitaxel [99]. This study showed evidence of both clinical benefit, with objective response rates of 50 % compared to historical controls, and immune activation, with increases in natural killer cells as well as durable effector-memory CD8⁺ T cell responses.

Some evidence suggests that patients treated with immune-based therapy and who subsequently require salvage chemotherapy may have an enhanced clinical response. One study of DCs transduced with a p53-expressing adenoviral vector in 29 patients with extensive-stage SCLC revealed that 60 % of patients developed p53-specific immunity, but all patients except one progressed on study [100]. Notably, about 60 % of patients with progressive disease—those with p53-specific immunity—demonstrated an objective response to salvage chemotherapy. Similarly, glioblastoma patients vaccinated with a dendritic cell vaccine who progressed on study also had a better than expected response to postvaccination salvage chemotherapy [101].

High-dose chemotherapy and immunotherapy

Several groups have also explored high-dose chemotherapy given with or without radiation as a platform for adoptive cellular therapy or vaccination. T cells specific for minor histocompatibility antigens were administered after allogeneic stem cell transplantation in one small 7 patient study [102]. The transferred T cells persisted for up to 21 days, and 5 of the 7 patients treated demonstrated complete but temporary remissions after adoptive T cell transfer. A larger clinical trial tested two types of conditioning followed by adoptive cellular therapy with autologous tumor-infiltrating lymphocytes (TIL) and IL-2 in 50 patients with metastatic melanoma [103]. High-dose cyclophosphamide and fludarabine alone resulted in a response rate of 49 %, and adding 2 or 12 Gray of total body irradiation resulted in response rates of 52 and 72 %, respectively. High serum levels of IL-7 and IL-15 were associated with lymphodepletion, and objective tumor responses correlated with longer telomere length of the transferred lymphocytes. High-dose chemotherapy followed by autologous stem cell transplantation as a platform for the administration of a GM-CSF-secreting, cell-based autologous tumor vaccine has been tested in at least 2 clinical trials that enrolled patients with acute myelogenous leukemia (AML), with an acceptable safety profile and evidence of vaccine-induced immune responses [82, 104].

Low-dose chemotherapy and immunotherapy

Immune modulating doses of chemotherapy (typically much lower than the standard doses used for cancer treatment) can be used to strategically enhance the activity of tumor vaccines in clinical trials rather than to kill cancer cells directly (Table 3). Several phase II clinical trials are historically important as they investigated the use of low-dose cyclophosphamide to relieve the inhibitory influence of suppressor T cells as they were defined in the 1970s and 1980s. In the aggregate, these early studies demonstrated that 300 mg/m² cyclophosphamide given 3 days prior to vaccination with a clustered carbohydrate antigen-keyhole limpet hemocyanin (KLH) vaccine resulted in higher antibody titers and longer survival than vaccination without cyclophosphamide pretreatment [105]. To definitely test these findings, a phase III clinical trial testing cyclophosphamide-modulated vaccination for metastatic breast cancer randomized 505 patients to treatment with 300 mg/m² cyclophosphamide followed by vaccination with KLH alone and 523 patients to treatment with 300 mg/m² cyclophosphamide followed by vaccination with clustered carbohydrate-KLH [106]. Although PFS and OS were no different for the two groups, an unplanned subset analysis suggested a possible trend toward improved PFS in patients in concurrent endocrine therapy.

The use of low-dose cyclophosphamide for immune modulation has subsequently been tested in several clinical trials. One study tested a GM-CSF-secreting, mesothelin-expressing cell-based tumor vaccine alone or given 1 day after cyclophosphamide at 300 mg/m² in 50 patients with metastatic pancreas cancer [107]. Although no definitive impact of chemotherapy was observed, there was a trend toward both increased mesothelin-specific CD8⁺ T cell responses and clinical benefit with cyclophosphamide-modulated vaccination as compared to vaccination alone.

A similar vaccine platform was given with 300 mg/m² cyclophosphamide to patients with advanced NSCLC, with a transient decrease in peripheral Tregs with time after cyclophosphamide observed [108]. A third study reported the activity of a peptide vaccine consisting of monocyte-derived DCs loaded with peptide epitopes specific for HER-2, hTERT, and PADRE alone or with 300 mg/m² cyclophosphamide given 2 days prior to vaccination in 11 patients with advanced ovarian cancer in remission [109]. The patients treated with cyclophosphamide-modulated vaccination had a trend toward improvement in survival compared to those treated with vaccination alone, and the 3-year OS for this small group of patients was 90 %. Adding cyclophosphamide to vaccination was associated with a transient decrease in neutrophils, but no change in peripheral lymphocytes or Tregs was observed. A recent randomized phase II clinical trial conducted in 68 patients with advanced renal cell carcinoma tested 17 vaccinations with a multi-peptide vaccine (IMA901), where the first vaccination was given 3 days after single dose of 300 mg/m² cyclophosphamide in 33 patients, and the 17 vaccinations were given with no pretreatment in a separate group of 35 patients [110]. Although there was no difference in PFS between the groups, there was trend toward longer OS in patients who received cyclophosphamide-modulated vaccination compared to those who received vaccination alone (median OS of 23.5 vs. 14.8 months). Interestingly, the immune response rates between the two groups were comparable, suggesting that cyclophosphamide pretreatment did not influence the induction of T cell responses. Furthermore, among immune responders, those pretreated with a single dose of cyclophosphamide had longer OS than those who were not pretreated with cyclophosphamide (HR 0.38, $p = 0.01$); there was no difference in survival between the arms in immune nonresponders (HR = 0.92, $p = 0.870$). In addition, those who developed multi-peptide immune responses survived longer than those who did not, suggesting an association of clinical benefit with a diverse immune response. The single dose of cyclophosphamide resulted in a roughly 20 % decline in Tregs at 3 days as compared to baseline. The percentage of proliferating cells among all regulatory T cells was also

decreased. There was no impact on absolute lymphocyte counts on either arm, and these effects were not observed in patients who received vaccination without cyclophosphamide. A distinct study tested a HER-2-positive, GM-CSF-secreting, cell-based breast tumor vaccine alone, or given in a specifically timed sequence with low, immune modulating doses of the two chemotherapy drugs cyclophosphamide and doxorubicin [111]. An innovative factorial response surface design was used to detect possible interactions between cyclophosphamide and doxorubicin, and the cyclophosphamide was given at doses of 0, 200, 250, or 350 mg/m² 1 day prior to vaccination, whereas the doxorubicin was given at doses of 0, 15, 25, or 35 mg/m² 7 days after vaccination. Vaccination alone induced new HER-2-specific delayed type hypersensitivity (DTH), with low levels of HER-2-specific antibodies also detected by ELISA. The addition of cyclophosphamide at 200 mg/m² maintained the DTH response and enhanced the HER-2-specific antibody response, whereas doses of cyclophosphamide at 250 or 350 mg/m² abrogated vaccine-induced immunity. Preliminary data suggest a decrement in peripheral Tregs that is dependent of the dose of cyclophosphamide. The chemotherapy dose combination that maximized vaccine-induced HER-2-specific antibody responses was 200 mg/m² cyclophosphamide and 35 mg/m² doxorubicin. Taken together, these data suggest there may be a narrow therapeutic window for the use of low-dose cyclophosphamide for immune modulation, particularly when used in sequence with low-dose doxorubicin.

Conclusions

A new era of effectively harnessing the immune system to treat and prevent cancer has begun. Two distinct immune-based therapies are now approved for cancer treatment: the first cancer vaccine (sipuleucel-T for advanced prostate cancer), and the first immune checkpoint inhibitor (ipilimumab for advanced melanoma). These early successes have led to heightened interest and activity in developing new strategies for tipping the balance of the host-tumor interaction toward definitive tumor rejection. It is clear that strategically integrating immune-based therapies with standard cancer treatment modalities, in particular chemotherapy drugs, has the potential to reengineer the overall host milieu and the local tumor microenvironment to disrupt pathways of immune tolerance and suppression. In designing combination immunotherapy regimens, clinical investigators should consider how chemotherapy impacts the immune system in order to guide the dose and schedule for integrating chemotherapy and immunotherapy. In particular, systematically defining the optimal drug dose and timing in relation to immune-based therapy in early-phase clinical studies is imperative for the design of phase II and III clinical trials with a higher likelihood of clinical success. Such thoughtful clinical development plans will accelerate the clinical development of chemoimmunotherapy regimens that result in a vigorous and sustained antitumor immune response that eradicates cancer and prevents its recurrence.

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References

1. Mellman I, Coukos G, Dranoff G. Cancer immunotherapy comes of age. *Nature*. 2011; 480:480–489. [PubMed: 22193102]
2. Kantoff P, Higano C, Shore N, Berger E, Small E, et al. Sipuleucel-T immunotherapy for castration-resistant prostate cancer. *N Engl J Med*. 2010; 363:411–422. [PubMed: 20818862]

3. Hodi F, O'Day S, McDermott D, Weber R, Sosman J, et al. Improved survival with ipilimumab in patients with metastatic melanoma. *N Engl J Med*. 2010; 363:711–723. [PubMed: 20525992]
4. Topalian SL, Hodi FS, Brahmer JR, Gettinger SN, Smith DC, et al. Safety, activity, and immune correlates of anti-PD-1 antibody in cancer. *N Engl J Med*. 2012; 366:2443–2454. [PubMed: 22658127]
5. Brahmer JR, Tykodi SS, Chow LQ, Hwu WJ, Topalian SL, et al. Safety and activity of anti-PD-L1 antibody in patients with advanced cancer. *N Engl J Med*. 2012; 366:2455–2465. [PubMed: 22658128]
6. Emens, L.; Jaffee, E. Immunotherapy and cancer therapeutics: why partner?. In: Prendergast, G.; Jaffee, E., editors. *Cancer immunotherapy and immunosuppression*. 1st edn.. Academic Press; Elsevier; London: 2007. p. 207-233.
7. Casares N, Pequignot MO, Tesniere A, Ghiringhelli F, Roux S, et al. Caspase-dependent immunogenicity of doxorubicin-induced cell death. *J Exp Med*. 2005; 202:1691–1701. [PubMed: 16365148]
8. Demaria S, Volm MD, Shapiro RD, Yee HT, Oratz R, et al. Development of tumor-infiltrating lymphocytes in breast cancer after neoadjuvant paclitaxel chemotherapy. *Clin Cancer Res*. 2001; 7:3025–3030. [PubMed: 11595690]
9. Emens LA, Machiels JP, Reilly RT, Jaffee EM. Chemotherapy: friend of foe to cancer vaccines? *Curr Opin Mol Ther*. 2001; 3:77–84. [PubMed: 11249735]
10. Green DR, Ferguson T, Zitvogel L, Kroemer G. Immunogenic and tolerogenic cell death. *Nat Rev Immunol*. 2009; 9:353–363. [PubMed: 19365408]
11. Apetoh L, Ghiringhelli F, Tesniere A, Obeid M, Ortiz C, et al. Toll-like receptor 4-dependent contribution of the immune system to anticancer chemotherapy and radiotherapy. *Nat Med*. 2007; 13:1050–1059. [PubMed: 17704786]
12. Ghiringhelli F, Apetoh L, Tesniere A, Aymeric L, Ma Y, et al. Activation of the NLRP3 inflammasome in dendritic cells induces IL-1-beta-dependent adaptive immunity against tumors. *Nat Med*. 2009; 15:1170–1178. [PubMed: 19767732]
13. Tesniere A, Schlemmer F, Boige V, Kepp O, Martins I, et al. Immunogenic death of colon cancer cells treated with oxaliplatin. *Oncogene*. 2010; 29:482–491. [PubMed: 19881547]
14. Vacchelli E, Galluzzi L, Rousseau V, Rigoni A, Tesniere A, et al. Loss of function alleles P2RX7 and TLR4 fail to affect the response to chemotherapy in non-small cell lung cancer. *Oncoimmunology*. 2012; 1:271–278. [PubMed: 22737602]
15. Van der Most RG, Currie AJ, Cleaver AL, Salmons J, Nowak AK, et al. Cyclophosphamide chemotherapy sensitizes tumor cells to TRAIL-dependent CD8⁺ T cell-mediated immune attack resulting in suppression of tumor growth. *PLoS ONE*. 2009; 4:e6982. [PubMed: 19746156]
16. Yang S, Haluska FG. Treatment of melanoma with 5-fluorouracil or dacarbazine in vitro sensitizes cells to antigen-specific CTL lysis through perforin/granzyme- and Fas-mediated pathways. *J Immunol*. 2004; 172:4599–4608. [PubMed: 15034078]
17. Ramakrishnan R, Assudani D, Nagaraj S, Hunter T, Cho HI, et al. Chemotherapy enhances tumor cell susceptibility to CTL-mediated killing during cancer chemotherapy in mice. *J Clin Invest*. 2010; 120:1111–1114. [PubMed: 20234093]
18. Michaud M, Martins I, Sukkurwala AQ, Adjeian S, Ma Y, et al. Autophagy-dependent anticancer immune responses induced by chemotherapeutic agents in mice. *Science*. 2011; 334:1573–1577. [PubMed: 22174255]
19. Zitvogel L, Kepp O, Senovilla L, Menger L, Chaput N, et al. Immunogenic tumor cell death for optimal anticancer therapy: the calreticulin exposure pathway. *Clin Cancer Res*. 2010; 16:3100–3104. [PubMed: 20421432]
20. Kaneno R, Shurin GV, Kaneno FM, Naiditch H, Huo J, et al. Chemotherapeutic agents in low noncytotoxic concentrations increase immunogenicity of human colon cancer cells. *Cell Oncol*. 2011; 116:222–233.
21. Correale P, Aquino A, Giuliani A, Pellegrini M, Micheli L, et al. Treatment of colon and breast carcinoma cells with 5-fluorouracil enhances expression of carcinoembryonic antigen and susceptibility to HLA-A*02/01-restricted, CEA-peptide-specific cytotoxic T cells in vitro. *Int J Cancer*. 2003; 104:437–445. [PubMed: 12584740]

22. Vereecque R, Saudemont A, Quesnel B. Cytosine arabinoside induces costimulatory molecule expression in acute myeloid leukemia cells. *Leukemia*. 2004; 18:1223–1230. [PubMed: 15152266]
23. Gebeh H, Lehe C, Barhoush E, Al-Romaih K, Aboussekhra A, et al. Doxorubicin downregulates cell surface B7–H1 expression and upregulates its nuclear expression in breast cancer cells: role of B7–H1 as an anti-apoptotic molecule. *Breast Cancer Res*. 2010; 12:R48. [PubMed: 20626886]
24. Donepudi M, Raychaudhuri P, Bluestone JA, Mokyr MB. Mechanism of melphalan-induced B7–1 gene expression in P815 tumor cells. *J Immunol*. 2001; 166:6491–6499. [PubMed: 11359799]
25. Sojka DK, Donepudi M, Bluestone JA, Mokyr MB. Melphalan and other anticancer modalities up-regulate B7-1 gene expression in tumor cells. *J Immunol*. 2000; 164:6230–6236. [PubMed: 10843675]
26. Adair SJ, Hogan KT. Treatment of ovarian cancer cell lines with 5′-aza-2′-deoxycytidine upregulates the expression of cancer-testis antigens and class I major histocompatibility complex-encoded molecules. *Cancer Immunol Immunother*. 2009; 58:589–601. [PubMed: 18791715]
27. Fonsatti E, Nicolay HJ, Sigalotti L, Calabro L, Pezzani L, et al. Functional upregulation of human leukocyte antigen class I antigens expression by 5′-aza-2′-deoxycytidine in cutaneous melanoma: immunotherapeutic implications. *Clin Cancer Res*. 2007; 13:3333–3338. [PubMed: 17545540]
28. Zou W, Restifo NP. T_H17 cells in tumour immunity and immunotherapy. *Nat Rev Immunol*. 2010; 10:248–256. [PubMed: 20336152]
29. Zou W. Regulatory T cells, tumor immunity, and immunotherapy. *Nat Rev Immunol*. 2006; 6:296–307.
30. Nizar S, Copier J, Meyer B, Bodman-Smith M, Galustian C, et al. T-regulatory cell modulation: the future of immunotherapy? *Br J Cancer*. 2009; 100:1697–1703. [PubMed: 19384299]
31. Ercolini AM, Ladle BH, Manning EA, Pfannenstiel LW, Armstrong TD, et al. Recruitment of latent pools of high-avidity CD8⁺ T cells to the antitumor immune response. *J Exp Med*. 2005; 201:1519–1602.
32. Ghiringhelli F, Larmonier N, Schmitt E, Parcellier A, Cathelin D, et al. CD4⁺CD25⁺ regulatory T cells suppress tumor immunity but are sensitive to cyclophosphamide which allows immunotherapy of established tumors to be curative. *Eur J Immunol*. 2004; 34:336–344. [PubMed: 14768038]
33. Lutsiak ME, Semnani RT, De Pascalis R, Kashmiri SV, Schlom J, et al. Inhibition of CD4⁺CD25⁺ T regulatory cell function implicated in enhanced immune response by low dose cyclophosphamide. *Blood*. 2005; 105:2862–2868. [PubMed: 15591121]
34. Taieb J, Chaput N, Scharz N, Roux S, Novault S, et al. Chemoimmunotherapy of tumors: cyclophosphamide synergizes with exosome based vaccines. *J Immunol*. 2006; 176:2722–2729. [PubMed: 16493027]
35. Ding ZC, Blazar BR, Mellor AL, Munn DH, Zhou G. Chemotherapy rescues tumor driven aberrant CD4⁺ T cell differentiation and restores an activated polyfunctional helper phenotype. *Blood*. 2010; 115:2397–2406. [PubMed: 20118405]
36. Machiels JP, Reilly RT, Emens LA, Ercolini AM, Lei R, et al. Cyclophosphamide, doxorubicin, and paclitaxel enhance the antitumor immune response of granulocyte/macrophage-colony stimulating factor-secreting whole-cell vaccines in HER-2/*neu* tolerized mice. *Cancer Res*. 2001; 61:3689–3697. [PubMed: 11325840]
37. Tseng CW, Hung CF, Alvarez RD, Trimble C, Huh WK, et al. Pretreatment with cisplatin enhances E7-specific CD8⁺ T cell-mediated antitumor immunity induced by DNA vaccination. *Clin Cancer Res*. 2008; 14:3185–3192. [PubMed: 18483387]
38. Banissi C, Ghiringhelli F, Chen L, Carpentier AF. Treg depletion with a low-dose metronomic temozolomide regimen in a rat glioma model. *Cancer Immunol Immunother*. 2009; 58:1627–1634. [PubMed: 19221744]
39. Chen CA, Ho CM, Chang MC, Sun WZ, Chen YL, et al. Metronomic chemotherapy enhances antitumor effects of cancer vaccine by depleting regulatory T lymphocytes and inhibiting angiogenesis. *Mol Ther*. 2010; 18:1233–1243. [PubMed: 20372107]
40. Hermans IF, Chong TW, Palmowski MJ, Harris AL, Cerundolo V. Synergistic effect of metronomic dosing of cyclophosphamide combined with specific antitumor immunotherapy in a murine melanoma model. *Cancer Res*. 2003; 63:8408–8413. [PubMed: 14679003]

41. Ghiringhelli F, Menard C, Puig PE, Ladoire S, Roux S, et al. Metronomic cyclophosphamide regimen selectively depletes CD4⁺CD25⁺ regulatory T cells and restores T and NK effector function in end stage cancer patients. *Cancer Immunol Immunother*. 2007; 56:641–648. [PubMed: 16960692]
42. Zhang L, Dermawan K, Jin M, Liu R, Zheng H, et al. Differential impairment of regulatory T cells rather than effector cells by paclitaxel-based chemotherapy. *Clin Immunol*. 2008; 129:219–229. [PubMed: 18771959]
43. Beyer M, Kochanek M, Darabi K, Popov A, Jensen M, et al. Reduced frequencies and suppressive function of CD4⁺CD25^{hi} regulatory T cells in patients with chronic lymphocytic leukemia after therapy with fludarabine. *Blood*. 2005; 106:2018–2025. [PubMed: 15914560]
44. Correale P, Cusi MG, Tsang KY, Del Vecchio MT, Marsili S, et al. Chemo-immunotherapy of metastatic colorectal carcinoma with gemcitabine plus FOLFOX4 followed by sub-cutaneous granulocyte-macrophage colony-stimulating factor and interleukin-2 induces strong immunologic and antitumor activity in metastatic colon cancer patients. *J Clin Oncol*. 2005; 23:8950–8958. [PubMed: 16061910]
45. Correale P, Tagliaferri P, Fioravanti A, Del Vecchio MT, Remondo C, et al. Immunity feedback and clinical outcome in colon cancer patients undergoing chemoimmunotherapy with gemcitabine + FOLFOX followed by subcutaneous granulocyte-macrophage colony-stimulating factor and aldesleukin (GOLFIG-1 trial). *Clin Cancer Res*. 2008; 14:4192–4199. [PubMed: 18593999]
46. Viaud S, Flament C, Zoubir M, Pautier P, LeCesne A, et al. Cyclophosphamide induces differentiation of Th17 cells in cancer patients. *Cancer Res*. 2011; 71:661–665. [PubMed: 21148486]
47. Moschella F, Valentini M, Aricò E, Macchia I, Sestili P, et al. Unraveling cancer chemoimmunotherapy mechanisms by gene and protein expression profiling of responses to cyclophosphamide. *Cancer Res*. 2011; 71:3528–3539. [PubMed: 21444678]
48. Gabrilovich DI, Nagaraj S. Myeloid-derived suppressor cells as regulators of the immune system. *Nat Rev Immunol*. 2009; 9:162–174. [PubMed: 19197294]
49. Salem ML, Al-Khami AA, El-Naggar SA, Díaz-Montero CM, et al. Cyclophosphamide induces dynamic alterations in the host microenvironments resulting in a Flt3 ligand-dependent expansion of dendritic cells. *J Immunol*. 2010; 184:1737–1747. [PubMed: 20083664]
50. Diaz-Montero CM, Salem ML, Nishimura MI, Garrett-Mayer E, Cole DJ, et al. Increased circulating myeloid-derived suppressor cells correlated with cancer stage, metastatic tumor burden, and doxorubicin-cyclophosphamide chemotherapy. *Cancer Immunol Immunother*. 2008; 58:49–59. [PubMed: 18446337]
51. Le HK, Graham L, Cha E, Morales JK, Manjili MH, et al. Gemcitabine directly inhibits myeloid-derived suppressor cells in BALB/c mice bearing 4T1 mammary carcinoma and augments expansion of T cells from tumor-bearing mice. *Int Immunopharmacol*. 2009; 9:900–909. [PubMed: 19336265]
52. Vincent J, Mignot G, Chalmin F, Ladoire S, Bruchard M, et al. 5-Fluorouracil selectively kills tumor-associated myeloid-derived suppressor cells resulting in enhanced T cell-dependent antitumor immunity. *Cancer Res*. 2010; 70:3052–3061. [PubMed: 20388795]
53. Kodumudi KN, Woan K, Gilvary DL, Sahakian E, Wei S, et al. A novel chemoimmunomodulating property of docetaxel: suppression of myeloid-derived suppressor cells in tumor bearers. *Clin Cancer Res*. 2010; 16:4583–4594. [PubMed: 20702612]
54. Kaneno R, Shurin GV, Tourkova IL, Shurin MR. Chemomodulation of human dendritic cell function by anti-neoplastic agents in low noncytotoxic concentrations. *J Transl Med*. 2009; 7:58. [PubMed: 19591684]
55. Shurin GV, Tourkova IL, Kaneno R, Shurin MR. Chemotherapeutic agents in noncytotoxic concentrations increase antigen presentation by dendritic cells via an IL-12-dependent mechanism. *J Immunol*. 2009; 183:137–144. [PubMed: 19535620]
56. Nakahara T, Uchi H, Lesokhin AM, Avogadri F, Rizzuto GA, et al. Cyclophosphamide enhances immunity by modulating the balance of dendritic cell subsets in lymphoid organs. *Blood*. 2010; 115:4384–4392. [PubMed: 20154220]

57. Wada S, Yoshimura K, Hipkiss EL, Harris TJ, Yen HR, et al. Cyclophosphamide augments antitumor immunity: studies in an autochthonous prostate cancer model. *Cancer Res.* 2009; 69:4309–4318. [PubMed: 19435909]
58. Schiavoni G, Mattei F, Di Puchio T, Santini SM, Bracci L, et al. Cyclophosphamide induces type 1 interferon and augments the number of CD44^{hi} T lymphocytes in mice: implications for strategies of chemoimmunotherapy of cancer. *Blood.* 2000; 95:2024–2030. [PubMed: 10706870]
59. Radojic V, Bezak KB, Skarica M, Pletneva MA, Yoshimura K, et al. Cyclophosphamide resets dendritic cell homeostasis and enhances antitumor immunity through effects that extend beyond regulatory T cell elimination. *Cancer Immunol Immunother.* 2010; 59:137–148. [PubMed: 19590872]
60. Salem ML, Díaz-Montero CM, Al-Khami AA, El-Naggar SA, Naga O, et al. Recovery from cyclophosphamide-induced lymphopenia results in expansion of immature dendritic cells which can mediate enhanced prime-boost vaccination antitumor responses in vivo when stimulated with the TLR3 agonist poly(I:C). *J Immunol.* 2009; 182:2030–2040. [PubMed: 19201856]
61. Salem ML, El-Naggar SA, Cole DJ. Cyclophosphamide induces bone marrow to yield higher numbers of precursor dendritic cells in vitro capable of functional antigen presentation to T cells in vivo. *Cell Immunol.* 2010; 261:134–143. [PubMed: 20036354]
62. Byrd-Leifer C, Block EF, Takeda K, Akira S, Ding A. The role of MyD88 and TLR4 in the LPS-mimetic activity of Taxol. *Eur J Immunol.* 2001; 31:2448–2457. [PubMed: 11500829]
63. Kawasaki K, Akashi S, Shimazu R, Yoshida T, Miyake K, et al. Mouse toll-like receptor 4.MD-2 complex mediates lipopolysaccharide-mimetic signal transduction by Taxol. *J Biol Chem.* 2000; 275:2251–2254. [PubMed: 10644670]
64. Wang J, Kobayashi M, Han M, Choi S, Takano M, et al. MyD88 is involved in the signal pathway for Taxol-induced apoptosis and TNF-alpha expression in human myelomonocytic cells. *British J Hematol.* 2002; 11:638–645.
65. Pfannenstiel LW, Lam SS, Emens LA, Jaffee EM, Armstrong TD. Paclitaxel enhances early dendritic cell maturation and function through TLR4 signaling in mice. *Cell Immunol.* 2010; 263:79–87. [PubMed: 20346445]
66. John J, Ismail M, Riley C, Askham J, Morgan R, et al. Differential effects of paclitaxel on dendritic cell function. *BMC Immunol.* 2010; 19:14–24. [PubMed: 20302610]
67. Chopra A, Kim TS, O-Sullivan I, Martinez D, Cohen EP. Combined therapy of an established, highly aggressive breast cancer in mice with paclitaxel and a unique DNA-based vaccine. *Int J Cancer.* 2006; 118:2888–2898. [PubMed: 16380982]
68. Eralp Y, Wang X, Wang JP, Maughan MF, Polo JM, et al. Doxorubicin and paclitaxel enhance the antitumor efficacy of vaccines directed against HER-2/*neu* in a murine mammary carcinoma model. *Breast Cancer Res.* 2004; 6:R275–R283. [PubMed: 15217493]
69. Chu Y, Wang LX, Yang G, Ross HJ, Urba WJ, et al. Efficacy of GM-CSF-producing tumor vaccine after docetaxel chemotherapy in mice bearing established Lewis lung carcinomas. *J Immunother.* 2006; 29:367–380. [PubMed: 16799332]
70. Prell RA, Gearin L, Simmons A, Vanroey M, Jooss K. The anti-tumor efficacy of a GM-CSF-secreting tumor cell vaccine is not inhibited by docetaxel administration. *Cancer Immunol Immunother.* 2006; 12:1–9.
71. Morre M, Beq S. Interleukin-7 and immune reconstitution in cancer patients: a new paradigm for dramatically increasing overall survival. *Target Oncol.* 2012; 7:55–68. [PubMed: 22383042]
72. Cho BK, Rao VP, Ge Q, Eisen HN, Chen J. Homeostasis-stimulated proliferation drives naïve T cells to differentiate directly into memory T cells. *J Exp Med.* 2000; 192:549–564. [PubMed: 10952724]
73. Goldrath AW, Bogatzki LY, Bevan MJ. Naïve T cells transiently acquire a memory-like phenotype during homeostasis-driven proliferation. *J Exp Med.* 2000; 192:557–564. [PubMed: 10952725]
74. Zitvogel L, Apetoh L, Ghiringhelli F, Kroemer G. Immunological aspects of cancer chemotherapy. *Nat Rev Immunol.* 2008; 8:59–73. [PubMed: 18097448]
75. Mackall CL, Bare CV, Granger LA, Sharrow SO, Titus JA, et al. Thymic-independent T cell regeneration occurs via antigen-driven expansion of peripheral T cells resulting in a repertoire that

- is limited in diversity and prone to skewing. *J Immunol.* 1996; 156:4609–4616. [PubMed: 8648103]
76. Gameiro SR, Caballero JA, Higgins JP, Apelian D, Hodge JW. Exploitation of differential homeostatic proliferation of T cell subsets following chemotherapy to enhance the efficacy of vaccine-mediated antitumor responses. *Cancer Immunol Immunother.* 2011; 60:1227–1242. [PubMed: 21544650]
 77. Borrello I, Sotomayor EM, Rattis FM, Cooke SK, Gu L, et al. Sustaining the graft versus-tumor effect through post-transplant immunization with granulocyte-macrophage colony-stimulating factor (GM-CSF)-producing tumor vaccines. *Blood.* 2000; 95:3011–3019. [PubMed: 10807763]
 78. Teshima T, Mach N, Hill GR, Pan L, Gillesen S, et al. Tumor cell vaccine elicits potent antitumor immunity after allogeneic T-cell-depleted bone marrow transplantation. *Cancer Res.* 2001; 62:796–800. [PubMed: 11830535]
 79. Luznik L, Slansky JE, Jalla S, Borrello I, Levitsky HI, et al. Successful therapy of metastatic cancer using tumor vaccines in mixed allogeneic bone marrow chimeras. *Blood.* 2003; 101:1645–1652. [PubMed: 12406877]
 80. Dudley ME, Wunderlich JR, Robbins PF, Yang JC, Hwu P, et al. Cancer regression and autoimmunity in patients after clonal repopulation with antitumor lymphocytes. *Science.* 2002; 298:2346–2357.
 81. Rapoport AP, Stadtmauer EA, Aqui N, Badros A, Cotte J, et al. Restoration of immunity in lymphopenic individuals by vaccination and adoptive T cell transfer. *Nat Med.* 2005; 11:1230–1237. [PubMed: 16227990]
 82. Borrello IM, Levitsky HI, Stock W, Sher D, Qin L, et al. Granulocyte-macrophage colony-stimulating factor (GM-CSF)-secreting cellular immunotherapy in combination with autologous stem cell transplantation (ASCT) as postremission therapy for acute myeloid leukemia. *Blood.* 2009; 114:1736–1745. [PubMed: 19556425]
 83. Kanakry CG, Hess AD, Gocke CD, Thoburn C, Kos F, et al. Early lymphocyte recovery after timed sequential chemotherapy for acute myelogenous leukemia: peripheral oligoclonal expansion of regulatory T cells. *Blood.* 2011; 117:608–617. [PubMed: 20935254]
 84. Von Mehren M, Arlen P, Gulley J, Rogatko A, Cooper HS, et al. The influence of granulocyte-macrophage colony-stimulating factor and prior chemotherapy on the immunological response to a vaccine (ALVAC-CEA-B7.1) in patients with metastatic carcinoma. *Clin Cancer Res.* 2001; 7:1181–1191. [PubMed: 11350882]
 85. Lutz E, Yeo CJ, Lillemoe KD, Biedrzycki B, Kobrin BJ, et al. A lethally irradiated allogeneic granulocyte-macrophage colony stimulating factor-secreting tumor vaccine for pancreatic adenocarcinoma: a phase II trial of safety, efficacy, and immune activation. *Ann Surg.* 2011; 253:328–335. [PubMed: 21217520]
 86. Emens LA. GM-CSF-secreting vaccines for solid tumors. *Curr Opin Investig Drugs.* 2009; 10:1315–1324.
 87. Small, E.; Demkow, T.; Gerritson, W., et al. A phase III trial of GVAX immunotherapy for prostate cancer in combination with docetaxel vs. docetaxel plus prednisone in symptomatic, castration-resistant prostate cancer (CRPC). *GU ASCO 2009.* 2009.
 88. Weihrauch MR, Ansen S, Jurkiewicz E, Geisen C, Xia Z, et al. Phase I/II combined chemioimmunotherapy with carcinoembryonic antigen-derived HLA-A2-restricted CAP-1 peptide and irinotecan, 5-fluorouracil, and leucovorin in patients with primary metastatic colorectal cancer. *Clin Cancer Res.* 2005; 11:5993–6001. [PubMed: 16115944]
 89. Kaufman HL, Lenz HJ, Marshall J, Singh D, Garrett C, et al. Combination chemotherapy and ALVAC-CEA/B7.1 vaccine in patients with metastatic colorectal cancer. *Clin Cancer Res.* 2008; 14:4843–4849. [PubMed: 18676757]
 90. Arlen PM, Gulley JL, Parker C, Skarupa L, Pazdur M, et al. A randomized phase II study of concurrent docetaxel plus vaccine versus vaccine alone in metastatic androgen-independent prostate cancer. *Clin Cancer Res.* 2006; 12:1260–1269. [PubMed: 16489082]
 91. Kyte JA, Gaudernack G, Dueland S, Trachsel S, Julsrud L, et al. Telomerase peptide vaccination combined with temozolomide: a clinical trial in stage IV melanoma patients. *Clin Cancer Res.* 2011; 17:4568–4580. [PubMed: 21586625]

92. Brunsvig PF, Kyte JA, Kersten C, Sundstrom S, Moller M, et al. Telomerase peptide vaccination in NSCLC: a phase II trial in stage III patients vaccinated after chemoradiotherapy and an update on a phase I/II trial. *Clin Cancer Res.* 2011; 17:6847–6857. [PubMed: 21918169]
93. Robert C, Thomas L, Bondarenko I, O'Day S, et al. Ipilimumab plus decarbazine for previously untreated melanoma. *N Engl J Med.* 2011; 364:2517–2526. [PubMed: 21639810]
94. Nistico P, Capone I, Palermo B, Del Bello D, Ferraresi V, et al. Chemotherapy enhances vaccine-induced antitumor immunity in melanoma patients. *Int J Cancer.* 2009; 124:130–139. [PubMed: 18839429]
95. Palermo B, Del Bello D, Sottini A, Serana F, Ghidini C, et al. Dacarbazine treatment before peptide vaccination enlarges T cell repertoire diversity of melan-A-specific, tumor-reactive CTL in melanoma patients. *Cancer Res.* 2010; 70:7084–7092. [PubMed: 20823160]
96. Alfaro C, Perez-Gracia JL, Suarez N, Rodriguez J, Fernandez de Sanmamed M, et al. Pilot clinical trial of type 1 dendritic cells loaded with autologous tumor lysates combined with GM-CSF, pegylated IFN, and cyclophosphamide for cancer patients. *J Immunol.* 2011; 187:6130–6142. [PubMed: 22048768]
97. Lynch TJ, Bondarenko I, Luft A, Serwatowski P, Barlesi F, et al. Ipilimumab in combination with paclitaxel and carboplatin as first-line treatment in stage IIIB/IV non-small cell lung cancer: results from a randomized, double-blind, multicenter phase II study. *J Clin Oncol.* 30:2046–2054. [PubMed: 22547592]
98. Reck M, Bondarenko I, Luft A, Serwatowski P, Barlesi F, et al. Ipilimumab in combination with paclitaxel and carboplatin as first-line therapy in extensive-disease small-cell lung cancer: results from a randomized, double-blind, multicenter phase II study. *Ann Oncol.* (epub ahead of print PMID: 22858559).
99. Brignone C, Gutierrez M, Mefti F, Brain E, Jarcau R, et al. First-line chemoimmunotherapy in breast carcinoma: combination of paclitaxel and IMP321 (LAG-3Ig) enhances immune responses and antitumor activity. *J Translat Med.* 2010; 23:71.
100. Antonia SJ, Mirza N, Fricke I, Chiappori A, Thompson P, et al. Combination of p53 cancer vaccine with chemotherapy in patients with extensive stage small cell lung cancer. *Clin Cancer Res.* 2006; 12:878–887. [PubMed: 16467102]
101. Wheeler CJ, Black KL, Liu G, Mazer M, Zhang XX, et al. Vaccination elicits correlated immune and clinical responses in glioblastoma multiforme patients. *Cancer Res.* 2008; 68:5955–5964. [PubMed: 18632651]
102. Warren EH, Fujii N, Akatsuka Y, Chaney CN, Mito JK, et al. Therapy of relapsed leukemia after allogeneic hematopoietic cell transplantation with T cells specific for minor histocompatibility antigens. *Blood.* 2010; 115:3869–3878. [PubMed: 20071660]
103. Dudley ME, Yang JC, Sherry R, Hughes MS, Royal R, et al. Adoptive cell therapy for patients with metastatic melanoma: evaluation of intensive myeloablative chemo-radiation preparative regimens. *J Clin Oncol.* 2008; 26:5233–5239. [PubMed: 18809613]
104. Ho VT, Vanneman M, Kim H, Sasada T, Kang YJ, et al. Biologic activity of irradiated, autologous, GM-CSF-secreting leukemia cell vaccines early after allogeneic stem cell transplantation. *Proc Natl Acad Sci USA.* 2009; 106:15825–15830. [PubMed: 19717467]
105. Emens LA, Jaffee EM. Toward a breast cancer vaccine: work in progress. *Oncology.* 2003; 17:1200–1211. [PubMed: 14569849]
106. Miles D, Roche H, Martin M, Perren TJ, Cameron DA, et al. Phase III multicenter clinical trial of the sialyl-TN (STn)-keyhole limpet hemocyanin (KLH) vaccine for metastatic breast cancer. *Oncologist.* 2011; 16:1092–1100. [PubMed: 21572124]
107. Laheru D, Lutz E, Burke J, Biedrzycki B, Solt S, et al. Allogeneic granulocyte macrophage colony-stimulating factor tumor immunotherapy alone or in sequence with cyclophosphamide for metastatic pancreatic cancer: a pilot study of safety, feasibility, and immune activation. *Clin Cancer Res.* 2008; 14:1455–1463. [PubMed: 18316569]
108. Schiller J, Nemunaitis J, Ross H, et al. A phase 2 randomized study of GM-CSF gene-modified autologous tumor vaccine (CG8123) with and without low dose cyclophosphamide in advanced stage non-small cell lung cancer (NSCLC). Presented at the International Association for Study of Lung Cancer. 2005

109. Chu CS, Boyer J, Schullery DS, Gimotty PA, Gamerman V, et al. Phase I/II randomized trial of dendritic cell vaccination with or without cyclophosphamide for consolidation therapy of advanced ovarian cancer in first or second remission. *Cancer Immunol Immunother.* 2011; 61:629–641. [PubMed: 22021066]
110. Walter S, Weinschenk T, Stenzl A, Zdrojowy R, Pluzanska A, et al. Multi-peptide immune response to cancer vaccine IMA901 after single dose cyclophosphamide associates with longer patient survival. *Nat Med.* 2012 doi:10.1038/nm.2883 (epub ahead of print).
111. Emens LA, Asquith JM, Leatherman JM, Kober BJ, Petrik S, et al. Timed sequential treatment with cyclophosphamide, doxorubicin, and an allogeneic granulocyte-macrophage colony-stimulating factor-secreting breast tumor vaccine: a chemotherapy dose-ranging factorial study of safety and immune activation. *J Clin Oncol.* 2009; 27:5911–5918. [PubMed: 19805669]

Table 1**Modulation of tumor cell immunogenicity by chemotherapy drugs**

Mechanism	Chemotherapy drugs	Tumor cell type	Reference
Sensitizes to TRAIL-dependent CD8 ⁺ T cell-mediated apoptosis	Cyclophosphamide	Mesothelioma cells	[15]
Sensitizes tumor cells to CD8 ⁺ T cell-mediated apoptosis through fas-, perforin-, or granzyme-mediated pathways	5-Fluorouracil Dacarbazine	Melanoma	[16]
Upregulation of mannose-6-phosphate receptors and increased permeability to granzyme-B released by CTLs	Paclitaxel Cisplatin Doxorubicin	Lymphoma Colon cancer cells	[17]
Induce autophagy, resulting in tumor-infiltrating DCs and T cells by released ATP	Mitoxantrone Oxaliplatin	Colon cancer cells	[18]
Induce immunogenic cell death through cell surface expression of calreticulin	Gemcitabine Cyclophosphamide Oxaliplatin Doxorubicin Paclitaxel	Colon cancer, melanoma, and fibrosarcoma cells	[19]
Increases expression of MHC class I and antigen-processing machinery components	Paclitaxel	Colon cancer cells	[20]
Expression of carcinoembryonic antigen (CEA)	5-Fluorouracil	Colon and breast cancer cells	[21]
Downregulation of B7-H1/PD-L1 from cell surface	Doxorubicin Ara-C (cytosine arabinoside)	Breast cancer and leukemia cells	[22, 23]
Increased expression of cell surface B7-1 and B7-2	Ara-C (cytosine arabinoside)	Leukemia cells Mastocytoma and plasma-cytoma cells	[23–25]
Upregulate expression of cancer–testis antigens	5′-aza-2′-deoxycytidine	Renal cell carcinoma, ovarian cancer, melanoma, glioma	[26, 27]

TRAIL tumor necrosis factor-related apoptosis-inducing ligand, *CTLs* cytotoxic T lymphocytes, *DCs* dendritic cells, *ATP* adenosine triphosphate, *MHC* major histocompatibility complex, *CEA* carcinoembryonic antigen, *PD-L1* programmed death ligand-1, *Ara-C* cytosine arabinoside

Table 2**Modulation of immunoregulatory cells by chemotherapy drugs**

Mechanism	Chemotherapy drugs
Decreased Treg number or function	Low dose
	Cyclophosphamide
	Standard dose
	Cyclophosphamide
	Paclitaxel
	Fludarabine
	Cisplatin
	Metronomic
	Cyclophosphamide
	Paclitaxel
Promote T helper type 1 immunity	Temozolamide
	Cyclophosphamide
	Paclitaxel
	Bleomycin
Promote T helper type 17 immunity	Melphalan
	Cyclophosphamide
Increase MDSCs	Cyclophosphamide
Decrease MDSCs	AC chemotherapy
	Gemcitabine
	5-Fluorouracil
	Cisplatin
	Docetaxel
Enhance DC function	Cyclophosphamide
	Vincristine
	Vinblastine
	Paclitaxel
	Docetaxel
	Methotrexate
	Mitomycin-C
	Doxorubicin
	5'-aza-2'-deoxycytidine

Treg = CD4⁺CD25⁺FoxP3⁺ regulatory T cells

MDSC myeloid-derived suppressor cells, AC doxorubicin/cyclophosphamide chemotherapy, DCs dendritic cells

Table 3

Clinical trials of chemoimmunotherapy with vaccines

Patient population	n	Vaccine	Chemotherapy drug	Immunologic outcome
<i>Concurrent standard-dose chemotherapy</i>				
Stage 2/3 pancreas cancer (ref [85])	60	GM-CSF-secreting, mesothelin ⁺ pancreas tumor cells	Vaccine once, then 5-fluorouracil-based chemoradiation, then 3 more vaccines	Induction of mesothelin-specific T cells, inhibited by chemoradiation, then restored by 3 vaccines
Stage 4 prostate cancer (ref [86, 87])	405	GM-CSF-secreting prostate tumor cells	Docetaxel 75 mg/m ² + vaccine versus + dexamethasone	Not reported, study terminated
Stage 4 colon cancer (ref [88])	118	ALVAC-CEA-B7.1	Standard 5-fluorouracil, leucovorin, irinotecan	No inhibition of CEA-specific immunity
Stage 4 colon cancer (ref [89])	17	CEA peptide	Standard 5-fluorouracil, leucovorin, irinotecan	50 % patients with new CEA-specific T cells
Stage 4 prostate cancer (ref [90])	28	PSA vaccinia/fowl pox	Standard docetaxel and dexamethasone	No inhibition of PSA-specific immunity
Stage 4 melanoma (ref [91])	25	GV1001 telomerase peptide	Temozolomide 200 mg/m ² daily ×5	78 % patients with new, durable GV1001-specific immunity
Stage 3 NSCLC (ref [92])	23	GV1001 telomerase peptide	Weekly docetaxel 20 mg/m ² + XRT,	80 % patients with new GV1001-specific immunity, 65 % durable
Stage 4 melanoma (ref [94, 95])	10	Melan-A/MART-1/gp-100 peptide	No dacarbazine versus dacarbazine 800 mg/m ² (n = 5 in each group)	Enhanced peptide-specific immunity with dacarbazine
Stage 4 cancers (ref [96])	24	Type 1 DCs + autologous tumor lysate + GM-CSF + peg-IFN	CY 600 mg/m ² D-7	36 % with new immunity
<i>Low-dose immunomodulatory chemotherapy</i>				
Stage 4 breast cancer (ref [106])	1,028	KLH (n = 505) KLH-STn (n = 523)	CY 300 mg/m ² each arm D-3	Not reported, no clinical difference
Stage 4 pancreas cancer (ref [107])	50	GM-CSF-secreting, mesothelin ⁺ pancreas tumor cells	No CY versus CY 300 mg/m ² D-1	Enhanced mesothelin-specific T cells with CY 300 mg/m ²
Stage 2–4 ovarian cancer (ref [109])	11	Peptide-loaded DCs: HER-2, hTERT, PADRE	No CY versus CY 300 mg/m ² D-2	Modest T cell responses to HER-2 and hTERT
Stage 4 renal cell carcinoma (ref [110])	68	Multipeptide vaccine IMA901	No CY versus CY 300 mg/m ² D-3	Enhanced peptide-specific responses on each arm, no difference in magnitude
Stage 4 breast cancer (ref [111])	28	GM-CSF-secreting, HER-2 ⁺ breast tumor cells	CY 0, 200, 250, 350 mg/m ² D-1 DOX 0, 15, 25, 35 mg/m ² D-7 Factorial response surface design of various dose combinations	Increased HER-2-specific immunity with doses of CY 200 mg/m ² + DOX 35 mg/m ²

ALVAC = canary pox virus; XRT = radiation therapy; MART-1 = melanoma antigen recognized by T cell; DCs = dendritic cells; STn = clustered carbohydrate antigen

GM-CSF granulocyte-macrophage colony-stimulating factor, CEA carcinoembryonic antigen, PSA prostate-specific antigen, NSCLC nonsmall cell lung cancer, IFN interferon, KLH keyhole limpet hemocyanin, D day, CY cyclophosphamide, DOX doxorubicin, hTERT human telomerase reverse transcriptase, PADRE pan DR-binding peptide

Table 4

Clinical trials of chemotherapy combined with immune checkpoint blockade

Patient population	<i>n</i>	Immune checkpoint modulator	Chemotherapy drug	Clinical outcome
Stage 4 metastatic melanoma (ref [93])	502	Ipilimumab (<i>n</i> = 251) Placebo (<i>n</i> = 251)	DTIC 850 mg/m ² each arm	Improved OS, and 1, 2, and 3 year survival with Ipilimumab + DTIC versus DTIC alone
Stage 4 NSCLC (ref [97])	204	Ipilimumab, concurrent versus phased	Paclitaxel 175 mg/m ² Carboplatin AUC 6	Improved immune-related and standard PFS with phased treatment with chemotherapy first, followed by chemotherapy with ipilimumab
Extensive-stage SCLC (ref [98])	130	Ipilimumab, concurrent versus phased	Paclitaxel 175 mg/m ² Carboplatin AUC 6	Improved immune-related but not standard PFS with phased treatment with chemotherapy first, followed by chemotherapy with ipilimumab
Stage 4 breast cancer (ref [99])	30	IMP321	Paclitaxel 80 mg/m ² D1, 8, 15 weekly	Clinical benefit of 90 % Objective response rate of 50 % versus 25 % for historical control group Increased APC number and activity, increased NK percentage, increased CD8 ⁺ memory T cells

DTIC dacarbazine, *IMP321* recombinant soluble LAG-3Ig fusion protein

OS overall survival, *NSCLC* nonsmall cell lung cancer, *AUC* area under the curve, *PFS* progression-free survival, *SCLC* small cell lung cancer, *APC* antigen-presenting cell, *NK* natural killer cell