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REVIEW

Combinatorial treatments including vaccines, chemotherapy and monoclonal antibodies for cancer therapy

Constantin N. Baxevanis · Sonia A. Perez · Michael Papamichail

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Abstract Accumulating evidence suggests that despite the potency of cytotoxic anticancer agents, and the great specificity that can be achieved with immunotherapy, neither of these two types of treatment by itself has been sufficient to eradicate the disease. Still, the combination of these two different modalities holds enormous potential for eliciting therapeutic results. Indeed, certain chemotherapeutic agents have shown immunomodulatory activities, and several combined approaches have already been attempted. For instance, chemotherapy has been proven to enhance the efficacy of tumor cell vaccines, and to favor the activity of adoptively transferred tumor-specific T cells. A number of mechanisms have been proposed for the chemotherapy-triggered enhancement of immunotherapy response. Thus, chemotherapy may favor tumor cell death, and by that enhance tumor-antigen cross-presentation in vivo. Drug-induced myelosuppression may induce the production of cytokines favoring homeostatic proliferation, and/or ablate immunosuppression mechanisms. Furthermore, the recently reported synergy between monoclonal antibodies and chemotherapy or peptide vaccination is based upon the induction of endogenous humoral and cellular immune responses. This would suggest that monoclonal antibodies may not only provide passive immunotherapy but can also promote tumor-specific active immunity. This article will review several strategies in which immunotherapy can be exploited in preclinical and clinical studies in combination with other agents and therapeutic modalities that are quite unique when compared with "conventional" combination therapies (ie, treatments with chemotherapeutic drugs or chemotherapy and radiotherapy based protocols). The results from these studies may have significant implications for the development of new protocols based on combinatorial treatments including vaccines, chemotherapy and monoclonal antibodies, suggesting an exciting potential for therapeutic synergy with general applicability to various cancer types. Given the complicity of immune-based therapies and cancer pharmacology, it will be necessary to bring together cancer immunologists and clinicians, so as to provide a robust stimulus for realizing the successful management of cancer in the near future.

Keywords Peptide vaccines · Cytotoxic drugs · Monoclonal antibodies · Combinatorial treatment · Chemotherapy · Immunotherapy

C. N. Baxevanis (✉) · S. A. Perez · M. Papamichail
Cancer Immunology and Immunotherapy Center,
St. Savas Cancer Hospital, 171 Alexandras Ave,
11522 Athens, Greece
e-mail: baxevanis@ciic.gr

Introduction

Conventional anti-cancer therapies (surgery and radio- and chemotherapy) have gained a considerable clinical success over the past years. Because of the limitations imposed by the given current treatments, tumor-free survival is not always accomplished. For instance, surgery and radiotherapy are quite effective in the treatment of localized tumors, but they usually play a palliative role in the treatment of disseminated diseases. Chemotherapy in these cases remains the treatment modality of choice, but severe toxic effects toward normal tissues often limit its use. The identification of tumor-specific antigens, tumor-specific lymphocytes, and tumor-specific T cell responses in cancer patients led to the development of immunotherapies aimed at augmenting antitumor immune responses. Studies mostly

performed in preclinical studies have indicated that both active and adoptive immunotherapies are quite effective against small tumor burdens, but seem to be incapable of controlling large tumor masses. The major limitation for combining antiblastic chemotherapy and immunotherapy is that cytotoxic drugs are generally regarded as immunosuppressive because of toxicity to the dividing immune cells in the bone marrow and peripheral lymphoid tissues.

However, there is now increasing evidence to suggest that the immune system can be activated variously by conventional therapies. Some therapeutic programmes can elicit specific cellular responses that render tumor-cell death immunogenic. Other drugs may have side effects that stimulate the immune system through transient lymphodepletion, by the subversion of immunosuppressive mechanisms or through direct or indirect stimulatory effects on immune effectors. Moreover, vaccination against cancer-specific antigens can sensitize the tumor to subsequent chemotherapeutic treatment. The challenge is to arm the host immune system so that it can control any residual disease. When taken together, the results from these studies indicate that there is a strong and developing case for combining chemotherapy and immunotherapy in cancer treatment. Together, the available information indicates that both conventional treatment and immunotherapeutic strategies might benefit from combined treatments aimed at controlling tumor growth, while allowing vaccine-induced immune responses to develop and eliminate the minimal residual disease.

Immunostimulatory properties of cytotoxic drugs

Drug-induced apoptosis

There is now increasing evidence that, under the right circumstances, chemotherapy-induced tumor-cell death can set the stage for an effective antitumor immune response. Anticancer drugs can induce apoptosis both by death-receptor-dependent and -independent pathways. Some anticancer drugs increase the expression of death receptors, including FAS, tumor-necrosis factor (TNF) receptors and TNF-related apoptosis inducing ligand receptors [50]. Other drugs do not alter expression of death receptors, but trigger apoptosis by inducing release of cytochrome *c* from mitochondria. Although chemotherapeutic drugs induce their primary damage in many different ways, most of them induce apoptosis not only in vitro but also in vivo [30, 41]. For example, a diverse set of agents, including cytarabine, mitoxantrone, etoposide and topotecan, have been shown to increase the number of apoptotic blasts in leukemia [34]. Importantly, the degree of apoptosis was found to correlate with clinical outcome for several different tumor types [11, 55]. Gemcitabine has been also shown to induce apoptosis of established tumors and through this to enhance the DC-dependent cross-presentation of tumor antigens to T cells [47, 48]. Human colon carcinoma cell lines that were treated with 5-fluorouracil acquired CD95 and ICAM1 expressions and became more sensitive to lysis by CTLs [62].

Lymphodepletion and adoptive immunotherapy

The onset of homeostatic T-cell proliferation via cytotoxic drug-induced lymphopenia has been described as a mechanism driving naive T cells to differentiate into memory ones [67]. Reconstituted T cell repertoire following lymphoablative treatment may be manipulated during vaccination towards a more robust tumor-specific immune response. In support of this, Hu et al. [29] reported that tumor-specific T cells preferentially expanded in tumor vaccine-draining lymph nodes after a melanoma vaccine given to RAG1 mice reconstituted with naive T cells from normal mice. T cells derived from reconstituted RAG1 hosts exhibited a higher level of melanoma-specific cytotoxicity in vitro. These cells were significantly more potent at mediating tumor regression in vivo after adoptive transfer into mice bearing established pulmonary metastases. In more clinically relevant models Teshima et al. [64], have tested a novel treatment strategy to stimulate specific antitumor activity against a solid tumor after bone marrow transplantation by vaccination with irradiated tumor cells engineered to secrete granulocyte macrophage colony-stimulating factor (GM-CSF). They found that vaccination was effective in stimulating potent and long-lasting antitumor activity in recipients of T-cell-depleted allogeneic bone marrow**.** This strategy may have implications in the treatment of patients with solid malignancies. The phenomenon of homeostatic proliferation was reported in patients with metastatic melanoma treated with in vitro expanded tumor-infiltrating lymphocytes (TILs) and IL-2 following a lymphodepleting non-myeloablative preparative regimen of cyclophosphamide and fludarabine [54]. This significant achievement was attributed to the key realization that the host's immune system needs to be properly conditioned, in order to create an appropriate lymphoid compartment that is devoid of regulatory mechanisms. Thus, lymphodepletion enables the host to accommodate transferred T lymphocytes and gives these cells an advantage over other competing cellular populations [44]. Moreover, lymphodepletion when combined with passive (ie, adoptive T cell transfer) or active (ie, vaccination) immunotherapy, may be most useful for generating effective antitumor responses [42].

Potentiation of antitumor immunity by chemotherapeutic drugs

It has been long recognized that some chemotherapeutic agents can modulate the immune response [51]. For example, pretreatment with agents such as cyclophosphamide had been shown to enhance the efficacy of adoptive transfer of antigen-specific lymphocytes [26]. Later on, a number of reports have demonstrated that some chemotherapeutic agents can enhance the antitumor activity of adoptively transferred T cells [5, 18, 28, 36, 40, 48]. The immunopotentiation of T cell-mediated immune response by cyclophosphamide has been suggested in various animal tumor models as well as in Phase I/II clinical trials [5–7, 18, 20, 23–25, 28, 36, 37, 40, 48]. The potentiation of tumor vaccines by cytotoxic drugs was first reported by Berd et al. [6, 7], who have showed that cyclophosphamide pretreatment markedly augments the development of DTH to melanoma-associated antigens, and that the resultant immunity can cause regression of metastatic tumors. In an other important study, Machiels et al. [36] found that cyclophosphamide, paclitaxel, and doxorubicin, when given in a defined sequence with a GM-CSF-secreting, HER-2/neu-expressing whole-cell vaccine, enhanced the vaccine's potential to delay tumor growth in HER-2/*neu* transgenic mice. Furthermore, paclitaxel and cyclophosphamide appeared to amplify the T helper 1 neu-specific T-cell response. These findings suggested that the combined treatment with immune-modulating doses of chemotherapy and the GM-CSF-secreting HER-2/neu vaccine can overcome immune tolerance and induce an antigen-specific antitumor immune response.

These data provided the immunological rationale for testing immune-modulating doses of chemotherapy in combination with tumor vaccines in patients with cancer. Another important finding was reported by Lutsiak et al. [35] who found that low doses of cyclophosphamide decrease the number and inhibitory function of CD4+ CD25+ regulatory T cells (Tregs) by downregulating the expression of key functional markers of Tregs, forkhead box P3 (FOXP3) and glucocorticoid-induced TNF-receptor-related protein (GITR). The effects of cyclophosphamide on Tregs and cyclophosphamide-stimulated type I IFN production [56] might account for the augmented antibody responses and the persistence of memory T cells. All these effects contribute to the eradication of immunogenic tumors in synergy with specific immunotherapies [19]. Other chemotherapeutics affecting vaccine-induced antitumor immunity include Gemcitabine that was found to induce apoptosis of established tumor cells in vivo, thereby increasing tumor antigen cross-presentation, leading to priming of tumor-specific CD8+ T cells [47]. Gemcitabine can also synergize with non-specific immunotherapy, mediated by CD40 ligation, to cure mice with established solid tumors [48]. This drug was also shown to have a selective detrimental effect on B lymphocytes inhibiting tumor-specific antibody production [46], which may skew antitumor immunity towards therapeutic T cell responses [53]. Moreover, Gemcitabine reduces the numbers of myeloid -derived suppressor cells potentiating antitumor immunity [60]. Consistent with this data, Gemcitabine can function in synergy with immunotherapeutic modalities to cure spontaneous tumors in HER-2/neu transgenic mice [31].

The combination of DC administration with repeated cycles of paclitaxel and dexamethasone (conditions similar to real clinical practice) resulted in the induction of antitumor immune response despite the immunosuppression induced by such a treatment [68]. This combined treatment led to a significant antitumor effect. The clinical success of this method may depend on careful selection of the dosage of the drugs to decrease chemotherapy-induced immunosuppression. In a mouse model, docetaxel when combined with a GM-CSF producing tumor vaccine, induced a significant tumor rejection and prolonged mice survival [12]. The administration of taxane-based adjuvant chemotherapy to women surgically treated for regional breast cancer was associated with higher phytohemmaglutinin-induced T-cell blastogenesis and NK cell lytic activity relative to a comparison group at long-term follow-up [9].

5-Flourouracil (5-FU) can sensitize breast and colon cancer cells to the cytotoxic effects of CEA peptide-specific CTL lines by increasing CEA expression in target cells [14, 15]. Similarly, when tested the immune sensitizing effects of 5-FU in vivo, Correale et al. [16] observed that this drug is able to enhance the antitumor activity of a thymidylate synthase (TS) polyepitopic peptide vaccine (TS/PP). Pathologic analyses of tumors from the vaccinated mice showed that lymphocytes infiltrated tumor cells and that expression of intracellular TS was reduced, which strongly suggested a vaccine-activated immune response. Because 5-FU treatment alone did not prevent or slow EL-4/HHD tumor growth in vivo, it seems likely that 5-FU may enhance the antitumor action of TS/PP vaccination by modulating TS expression in these target cells. Most recently, this group has found that the combination of 5-Fu with gemcitabine and oxaliplatin potentiated the killing efficacy of tumor peptide-specific CTL only when added at a later time-point after the in vitro stimulations with the cognate peptide [17].These results were translated in an in vivo therapeutic model where HLA-A2.1 transgenic mice with established syngeneic tumors showed prolonged survival and high cure rates when receiving a peptide vaccine and a multidrug chemotherapy administered late after vaccination [17]. Furthermore, 5-FU combined with cisplatin was investigated for the in vivo antitumor effects of intratumoral administration of dendritic cells after low-dose chemotherapy using

these two drugs. Combination of injection of dendritic cells and systemic chemotherapy induced complete rejection of the treated tumor. Moreover, the antitumor effects were also observed on a distant tumor inoculated in the contralateral flank of the animal [62].

5-Aza-2′-deoxycitydine (DAC) is a potent and specific inhibitor of DNA methyltransferase able to reinduce gene expression and cellular differentiation by mechanism involving DNA hypomethylation [52, 61]. DAC was demonstrated to restore expression of HLA class I genes frequently switched off during cancer progression and to abolish resistance of tumor cells to lysis by antigen-specific cytotoxic T lymphocytes [59]. Expression of tumor-associated antigens such as MAGE, GAGE, BAGE, SSX, and LAGE-1/NY-ESO-1 can be easily restored in tumor cells after treatment with DAC [8, 33, 58]. Because DAC facilitates signal transduction through IFN-γ receptor [21] and because of its multiple immunomodulatory actions, Kozar et al. [32] evaluated the antitumor effectiveness of this methylation inhibitor with IL-12, a cytokine that induces tumor regression through induction of IFN-γ secretion [45].Treatment with DAC or IL-12 given alone produced moderate antitumor effects. Combined treatment resulted in potentiated antitumor effects and produced 70% long-term survivors among mice inoculated with L1210 leukemia cells.

Potentiation of cellular immune responses in cancer patients by anthracyclines has been studied for decades [2, 49]. Moreover, effective chemo-immunotherapy of L1210 leukemia cells in vivo was achieved using doxorubicin combined with IL-12 [69]. In a more recent report [10], tumor cells dying in response to anthracyclins were shown to elicit an effective antitumor immune response that suppressed the growth of inoculated tumors or led to the regression of established neoplasia. In this model, anthracyclin-induced apoptosis via caspase activation was immunogenic. Caspase inhibition by Z-VAD-fmk or transfection with the baculovirus inhibitor p35 did not inhibit anthracyclin (doxorubicin)-induced cell death, yet suppressed the immunogenicity of dying tumor cells in several rodent models of neoplasia. Freshly excised tumors became immunogenic upon doxorubicin treatment in vitro, and intratumoral inoculation of doxorubicin could trigger the regression of established tumors in immunocompetent mice. These results delineated a procedure for the generation of cancer vaccines and the stimulation of anti-neoplastic immune responses in vivo.

Combining chemotherapy with vaccination in clinical trials

The in vivo preclinical data by Machiels et al. [36] addressing the issue of chemotherapy and cancer vaccines indicated that taxane therapy (employing taxol) may in fact increase T-cell precursors rather than deplete them. Later on, Arlen et al. [3] designed one phase II study of patients with metastatic androgen-inhependent prostate cancer randomized to receive vaccine either alone or with low-dose docetaxel. The vaccination regimen was composed of: (a) recombinant vaccinia virus (rV) that expresses the prostate-specific antigen gene (rV-PSA) admixed with (b) rV that expresses the B7.1 costimulatory gene (rV-B7.1), and (c) sequential booster vaccinations with recombinant fowlpox virus (rF-) containing the PSA gene (rF-PSA).

Patients received granulocyte macrophage colony-stimulating factor with each vaccination. The median increase in T-cell precursors to PSA was equal in both arms following 3 months of therapy. In addition, immune responses to other prostate cancer-associated tumor antigens were also detected postvaccination. Eleven patients who progressed on vaccine alone crossed over to receive docetaxel at time of progression. Median progression-free survival on docetaxel was 6.1 months after receiving vaccine when compared with 3.7 months with the same regimen in a historical control. This was the first clinical trial to show that docetaxel can be administered safely with immunotherapy without inhibiting vaccine specific T-cell responses. Based on their findings, the authors hypothesized that patients previously vaccinated with an anticancer vaccine may respond longer to docetaxel compared with a historical control of patients receiving docetaxel alone. In another phase I study [27], 17 patients with different types of advanced cancer were immunized with antigen cytochrome P450 1B1 (CYP1B1), which is overexpressed in almost all human tumors [38]. Six patients developed immunity to CYP1B1, three of whom developed disease stabilization. All but 1 of the 11 patients who did not develop immunity to CYP1B1 progressed and did not respond to salvage therapy. Five patients who developed immunity to CYP1B1 required salvage therapy for progressive metastatic disease and showed marked response to their next treatment regimen, most of which lasted longer than 1 year.

Antonia et al. [1] vaccinated 29 patients with extensive stage small cell lung cancer, with a DC-based p53 vaccine.Objective clinical response to vaccination as well as subsequent chemotherapy was evaluated. p53-specific T cell responses to vaccination were observed in 57.1% of patients. One patient showed a clinical response to vaccination, whereas most of the patients had disease progression. However, they observed a high rate of objective clinical responses to chemotherapy (61.9%) that immediately followed vaccination. Clinical response to subsequent chemotherapy was closely associated with induction of immunologic response to vaccination. Furthermore, Wheeler et al. [66] analyzed survival and progression times in 25 vaccinated (13 with and 12 without subsequent chemotherapy)

and 13 nonvaccinated de novo glioblastoma (GBM) patients receiving chemotherapy. Vaccinated patients receiving subsequent chemotherapy exhibited significantly longer times to tumor recurrence after chemotherapy relative to their own previous recurrence times, as well as significantly longer postchemotherapy recurrence times and survival relative to patients receiving isolated vaccination or chemotherapy. These data suggest that vaccination against cancer-specific antigens can sensitize the tumor against subsequent chemotherapeutic treatment. Although the mechanisms that underlie such a synergistic effect have not been elucidated, we speculate that the vaccination-induced increase in the frequency of primed T cells, although by itself irrelevant for tumor progression, may constitute a major advantage as soon as the tumor is insulted by cytotoxic drugs.

Combinatorial treatments utilizing monoclonal antibodies

Recent reports have provided strong evidence for the role of the Fc domain in the efficacy of antitumor mabs. In particular, Fcγ receptors have been demonstrated to significantly modulate the biologic activity of IgG anti-HER-2/neu antibodies in mouse/tumor models [13]. Moreover, a positive correlation between the presence of FcγRIIa and FcγRIIIa allelels and the outcome of rituximab combined with chemotherapy has been found in patients with malignant hematological diseases [64, 65]. These studies have suggested that the interaction between Fc and Fcγ receptors is critical to antibody efficacy and is correlative with the clinical outcome in patients. Indeed, in a recent study [4], the combination of trastuzumab plus taxanes, but not taxanes alone, induced a recruitment of natural killer cells to tumor sites, providing supportive evidence for the involvement of ADCC by Fcγ receptor-bearing effector cells in situ. Furthermore, treatment with trastuzumab plus paclitaxel exhibited enhanced endogenous humoral and cellular anti-HER-2/neu responses, which associated with favorable clinical outcomes in patients with advanced breast cancer [63]. The combination of mab treatment and peptide vaccination may also have a potential clinical utility. To this end, it has been recently reported that antibody-mediated internalization of HER-2/neu receptors increases the sensitivity of HER-2/neu expressing tumor cells by PBMCs stimulated with HER-2/neu-derived vaccine peptides [43]. In the same study, it was shown that PBMCs from patients who have been vaccinated with HER-2/neu peptides were more sensitive to the effects of trastuzumab on autologous breast tumor cells (even if the latter expressed low levels of HER-2/neu). Schuurhuis et al. [57] have recently reported that immune complexes (IC)-loaded DCs induce a remarkable protective immunity against tumors expressing the antigen present in the IC. Based on this finding, it is reasonable to suggest that uptake of HER-2/neu-trastuzumab complexes by DCs in vivo may result in more efficient CD8 HER-2/neu-specific responses. Cellular vaccines combined with monoclonal antibodies also offer an attractive approach for cancer treatment. To this end, promising preliminary results of ipilimumab in combination with a whole tumor-cell vaccine have been seen in patients with asymptomatic androgen-independent prostate cancer [22]. Chemotherapy naive patients were treated with a GM-CSF transduced allogeneic prostate cancer cell line vaccine (GVAX; Cell Genesys, South San Francisco, CA) every 2 weeks and with ipilimumab. The combination therapy resulted in a greater proportion of patients with declines in PSA and increased cellular immunity to the vaccine, than seen with vaccine alone. Clinical trials using ipilimumab combined with a peptide vaccine showed antitumor activity in patients with melanoma, though severe but reversible immune breakthrough events were seen [39]. The induction of endogenous cellular and humoral immunity through the combinatorial treatment with mabs and chemotherapy or vaccines (peptide- or cell-based vaccines) suggests that therapeutic antibodies not only provide passive immunotherapy through ADCC but also can promote active immunity resulting in augmentation of immunologic memory. This may dramatically increase the clinical utility and potential therapeutic benefit of antibody treatment when combined with other modalities (Table 1).

Table 1 Chemotherapy can augment immunotherapy by multiple pathways

	Drugs	References
1. Increased antigen cross-presentation	Gemcitabine	[33, 47, 48]
2. Activation of dendritic cells	Paclitaxel	[47, 48, 69]
3. Homeostatic proliferation	Alkylating agents, fludarabine	[42, 49, 54]
4. Increased homing to tumors	Gemcitabine, antivascular flavonoids	[33, 48, 62]
5. Inhibition of immunosuppressive cells	Gemcitabine, cyclophosphamide, taxanes	[31, 35, 42, 47, 48, 56, 60]
6. Upregulation of recognition molecules	Cisplatin, 5-FU, 5-aza-2′ deoxycitidine	[33, 42, 50, 59]

Conclusion

Despite major advances in the field of cancer immunotherapy and, in particular, in vaccine technology, the outcomes from clinical trials are far inferior to that anticipated. Accumulating evidence suggests that although vaccines exist with potent antitumor potential, still vaccinated patients are not in the position to mount robust vaccine-specific immune responses.However, many experimental studies and a few clinical trials have suggested that standard chemotherapy may synergize with active immunization for eliciting more effective antitumor immunity. The scenario of such combinatorial treatments is that vaccination towards tumor-associated antigens can sensitize the tumor against subsequent therapy with cytotoxic drugs although the reciprocal situation may also be true (i.e., chemotherapy preceding immunotherapy). At present, conclusive information is lacking about the exact mechanisms responsible for the chemotherapy-mediated potentiation of antitumor immune responsiveness. Nevertheless, in general, we may speculate that chemotherapy, by disrupting tolerogenic mechanisms and destructing tumor cells, paves the way for the vaccine-specific T cell clones to mediate more efficient antitumor responses. To this end, it is worth mentioning that the compromised immunity in patients with advanced cancer provides a serious obstacle for successful treatment. This holds true even in cases where conventional anticancer therapies are being used to generate minimal residual disease for further treatment with immunotherapy. With this consideration in mind it is reasonable to propose the application of combinatorial treatments for cancer therapy at earlier stages of tumorigenesis. Furthemore the precise characterization of immunoregulatory circuits that form the platform for therapeutic synergy will clear the paths for rationally designed combinatorial vaccine trials and will bring scientists and clinicians together in the fight against cancer.

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