**REVIEW**

# Novel mechanism of synergistic effects of conventional chemotherapy and immune therapy of cancer

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**Abstract** There is mounting evidence to support the use of a combination of immunotherapy with chemotherapy in the treatment of various types of cancers. However, the mechanism(s), by which these modalities are synergized, are not fully understood. In this review, we discuss several possible mechanisms of the combined effect of immunotherapy and chemotherapy of cancer. We will examine various aspects of this issue such as the combination of different treatment options, the dosage for each arm of treatment, and, more importantly, the timing and sequence of the administration of these treatments.

**Keywords** Chemotherapy · Immunotherapy · Combination therapy · Autophagy · Apoptosis

## Introduction

The notion that the successful treatment of cancer will require a combination of different modalities like surgery, radiation therapy, chemotherapy, and targeted therapy is widely accepted. Until recently, immunotherapy was not a part of this roster, due to a perceived lack of clinical efficacy and the surmised incompatibility with immune-suppressive chemotherapy. The situation has changed with an accumulation of data on the clinical effects of several cancer vaccines and the check point blockade with CTLA4 and PD1 antibodies. Adoptive T-cell therapy has demonstrated a therapeutic promise in patients with metastatic melanoma. The main limitation of these approaches is that the responses are restricted to a relatively small proportion of patients. This could be due to the effect of various immunosuppressive factors including regulatory T cells, myeloid-derived suppressor cells, inhibitory cytokines and receptors expressed by tumor cells, the varying ability of cytotoxic T cells to penetrate tumor parenchyma and recognize tumor-associated antigens, the correct choice of antigen for immunization, etc. A combination of therapies that can overcome immunosuppression, improve cross-presentation of tumor antigens, and support T cell proliferation, as well as their better penetration of tumor parenchyma, would be the ideal treatment scenario. A number of phase I/II clinical trials reported that a combination of chemotherapy with different cancer vaccines resulted, unexpectedly, in substantially improved clinical responses [1]. It has been shown that single doses of some chemotherapeutic drugs induce an antitumor response by causing immunogenic cell death [2]. Chemotherapy has also been shown to render cancer cells more susceptible to killing by CTLs [3]. However, repeated doses of chemotherapy induce immune suppression in mice [4]. The same is true for many chemotherapeutics in cancer patients [5]. Standard treatment with paclitaxel (TAX) was shown to inhibit macrophage, NK cells and effector T cells: all of which are important for tumor rejection [6]. These data raise the question of how conventional chemotherapy can be effectively combined with immunotherapy in treating cancer. This question could not be answered without a clear understanding of the mechanism of the combined effect of these two therapeutic modalities.

## Impact of chemotherapy on immune cells

Chemotherapy is known to affect different cells of the immune system. It appears that its effect depends on the

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dosage of the drugs. A number of chemotherapeutic drugs, when administered at low doses, were shown to augment dendritic cell (DC) functions. Cyclophosphamide, vincristine, and TAX all have well-documented effects on DC maturation and function [7–10]. Pro-inflammatory CD4$^+$ effector T cells are critical factors in effective antitumor response [11, 12]. Polyfunctional, activated CD4$^+$ effector T cells, following chemotherapy, have a central role in strengthening and sustaining the overall host antitumor immunity, and the outcome of antitumor immune response. The alkylating agent, cyclophosphamide, in combination with adoptive cell therapy (ACT) [13] has been shown to be very effective in driving the effector development of tumor-specific CD4 + T cells. Chemotherapy can influence the tumor microenvironment by modulating the expression of tumor antigens, accessory molecules of T cell activation or inhibition, and those involved in antigen processing and presentation. Paclitaxel induces cytokine production patterns, typical of the T-helper type I phenotype, thereby promoting effective CTL responses. Chemotherapy can improve the penetration of CTLs into the tumor parenchyma [14]. The success of a partnership between chemotherapy and immunotherapy also relies on the capacity of the antigen-presenting cells, like DC, to engulf-dying tumor cells and then to process and present tumor antigens to naive and/or central memory T cells. "eat me" signals, as well as antigen transfer from dying/damaged tumor cells, may not only regulate antigen uptake, but also the antigen processing, presentation, and co-stimulation of APCs [15]. This effect can be mediated by heat shock proteins (HSPs) [16]. The NKG2D ligand on the NK cells and death receptors, like Fas, are also up-regulated by signals from the dying tumor cells [17]. The role of the chromatin-associated and damage-associated molecular pattern molecule HMGB1, released by dying tumor cells in the activation of toll-like receptors (TLR) 2 and 4, has been demonstrated [18].

In addition, chemotherapy can be used to manipulate systemic pathways of immune tolerance and regulation. To illustrate the point, treatment of HER2 (Human epidermal growth receptor 2) transgenic mice with TAX has been shown to increase the potency of tumor vaccines that express HER2 and GM-CSF [19]. The anthracycline, doxorubicin (DOX), has been known to enhance the antitumor efficacy of GM-CSF-transfected vaccines [20].

Chemotherapeutic drugs initiate a series of cellular responses that make tumor cells more immunogenic. Anthracycline-treated tumor cells induce an immune response via translocating calreticulin to the cell surface, which emits an "eat me" signal for DC. This translates into activation of tumor-specific T cell responses. [21]. Here, we will not be discussing the effect of chemotherapy on immunogenicity of tumors, since it was addressed in several recent reviews [22, 23].

In recent years, accumulated evidence pointed out the possible effect of chemotherapy on regulatory immune cells. Suppressor cells of the immune system, like myeloid-derived suppressor cells (MDSC) and regulatory T cells (Tregs), control immune responses in cancer [24, 25]. Chemotherapy has been shown to have an impact on both of these cell types. The anti-metabolite drug, gemcitabine, eliminates MDSC in murine tumor models, thereby enhancing the activity of CD8$^+$ T cells and NK cells [26, 27]. Cisplatin (CIS), given prior to DNA vaccines encoding calreticulin (CRT), can decrease levels of peripheral MDSC in tumor-bearing mice [28]. In non-small cell lung cancer (NSCLC) patients, TAX decreases Tregs, selectively through Fas-mediated apoptosis, and up-regulates the T-helper type 1 cytokines IFN-γ and IL-2, and CD44 in CD4$^+$ and CD8$^+$ effector T cells [29]. Another anti-metabolite, fludarabine, has been known to decrease the function and total number of Tregs in B-cell chronic lymphocytic leukemia patients [30]. 5-FU can selectively kill MDSC without a significant drop in the other cells of the immune system. The administration of 5-FU also restored the capacity of intratumoral CD8 + T cells to produce IFN-γ and synergistically led to suppression of tumor progression [31]. More recently V. Bronte's group has shown that 5-FU when administered prior to antigen-specific adoptive CD8 + T cell transfer led to significant tumor regression in a MCA203 murine model. 5-FU depleted splenic MDSC but did not cause immunogenic death of tumor cells [32]. Metronomic chemotherapy, the chronic administration of chemotherapeutic agents at relatively low, minimally toxic doses with no prolonged drug-free breaks, yielded surprisingly favorable results [33, 34]. There have been encouraging reports with different metronomic treatment regimens in various recurrent cancers like ovarian cancer [35], hormone-resistant prostate cancer [36], and metastatic melanoma [37]. It was suggested that this type of chemotherapy inhibited tumor growth, primarily by targeting the tumor vasculature instead of the tumor cells, while significantly reducing undesirable toxic side effects and helping in overcoming drug resistance [38] However, it appears that metronomic chemotherapy may have a direct effect on the immune system as well. Low doses of anti-cancer drugs have been shown to enhance antitumor immune response and increase the efficacy of immunotherapy [39]. Iterative low dosing of cyclophosphamide, in late stage cancer patients, significantly depleted Tregs and enhanced NK and T cell effector functions [40]. In a murine model of glioma, low-dose metronomic temozolomide treatment resulted in the inhibition and depletion of the immunosuppressive activity of Tregs [41] Tanaka and his colleagues found that chemotherapeutic drugs like vinblastine, TAX, and etoposide, when used in metronomic chemotherapy regimens, could promote dendritic cell maturation at non-toxic concentrations [42].

Thus, ample evidence pointed to a positive effect on chemotherapy on the immune system. However, most of those data were obtained, either using single dose or low non-toxic doses of chemotherapeutics. In many instances, conventional doses of chemotherapy, that require repeated cycles of treatment, cause substantial immune suppression and toxic side effects [4, 43]. This suggested that other mechanisms could be involved, when conventional chemotherapy is combined with immune therapy of cancer.

## Apoptosis and autophagy as the mechanism of chemotherapy

Most chemotherapeutic drugs have been shown to induce apoptosis of tumor cells. Apoptosis involves activation of catabolic enzymes that leads to the destruction of cell organelles and, ultimately, cell death. Autophagy is a complex process that cells use to avoid cell death (and suppress apoptosis). In some settings, it constitutes an alternative cell-death pathway. Under the influence of an external stimulus like radiation, nutrient stress or chemotherapy, autophagy is initiated by the formation of a phagophore around cytoplasmic oraganelles and/or some portion of the cytosol. The autophagic pathway involves 5 main molecular components (1) the Atg1/unc-51-like kinase (ULK) complex; (2) the Beclin 1/class III phosphatidylinositol 3-kinase (PI3K) complex; (3) two transmembrane proteins, Atg9 and vacuole membrane protein 1 (VMP1); (4) two ubiquitin-like protein (Atg12 and Atg8/LC3) conjugation systems; and (5) proteins that mediate fusion between autophagosomes and lysosomes. The enclosed material is sequestered in a vacuole lined by two membranes called the autophagosome. Autophagosomes then undergo fusion with either endosomes or lysosomes [44]. The autophagic process is regulated by both class I and class III phosphatidylinositol 3-kinase (PI3K) pathways [45].

There is now enough evidence pointing on the ability of different types of cancer therapy, including chemotherapy, to induce autophagy [46]. Some examples include the effect of temozolomide (TMZ), which induced autophagy, but not apoptosis in malignant glioma cells. Treatment of the cells with TMZ caused the incorporation of LC3 on autophagosome membrane. Blocking of autophagy by 3-MA inhibited LC3 localization to the autophagosomal membrane and also suppressed the anti-tumor effect of TMZ [47]. Arsenic trioxide inhibited cell division and induced cell death by autophagy, characterized by the appearance of acidic vesicular organelles, in human glioma lines [48]. Tamoxifen induced a time-dependent accumulation of autophagic vacuoles in MCF-7 breast tumor cells, through the down-regulation of protein kinase B [49].

Apoptosis and autophagy are intricately connected. Both autophagy and apoptosis can be triggered by common upstream signals. Several pro-apoptotic signals such as TRAIL, TNF, and FADD also induce autophagy. Bcl-2 inhibits Beclin-1-dependent autophagy, thereby functioning as a pro-survival and an anti-autophagic regulator. Beclin-1 has been shown to be the substrate for death-associated protein kinase [50] which can induce apoptosis. Many Atg proteins can be cleaved by caspases that are activated during apoptosis [51].

Controversy exists as to whether autophagy kills cancer cells or sustains their survival under stressful conditions. The nutrient recycling functions of autophagy promote cell survival, whereas a high level of autophagy promotes its pro-death function. Inactivation of autophagy-specific genes, such as beclin 1, results in an increased tumorigenesis in mice, and the over-expression of such genes (beclin 1, Atg5) inhibits the formation of human breast tumors in mouse models [52, 53]. The Hypoxia-inducible factor 1α (HIF-1α), a key transcription factor of angiogenesis, invasion, and metastasis in hypoxic tumors is a positive regulator of autophagy [54]. The serine/threonine kinase, mTOR, is a negative regulator of autophagy, and recent findings point to its potential involvement in controlling the proliferation, survival, and death of cancer cells [55].

Autophagy has now been shown to be associated with several major events, involving cells of the immune system. Autophagy has been demonstrated to be up-regulated at the immunological synapse, during DC–T cell contact [56]. DC also use autophagy to promote cross-presentation of tumor antigens on MHC class I complexes for cytotoxic T-lymphocyte (CTL) activation and to facilitate antigen expression on MHC class II molecules for T-helper (Th) cell activation [57]. Autophagy is up-regulated in Th2 CD4$^+$ T cells, more than in Th1 cells, and is important for the survival of a Th2 cell line, upon growth factor withdrawal [58], and is required for the survival of mature T cells, once they migrate to the periphery [59]. The role of autophagy vastly depends on the cell type and stimuli received, and it is now understood that blocking autophagy can skew the balance of immune subsets.

## Autophagy as the mechanism of synergy between chemotherapy and immune therapy of cancer

*In vitro* experiments have demonstrated that pre-treatment of tumor cells, with different chemotherapeutic drugs (TAX, DOX, and CIS), can sensitize tumor cells to antigen-specific killing by activated CTLs. This effect was mediated via up-regulation of cation-independent mannose-6-phosphate receptors (MPR) on the surface of tumor

cells [60]. The multi-functional, ∼300 kDa MPR, along with the 46-kDa cation-dependent mannose-6-phosphate receptor, is responsible for the binding and uptake of mannose-6-phosphate-containing molecules [61]. Inside the cell, MPR transport the ligand-receptor complex from the trans-golgi network (TGN) to endosomes, where the ligands are subsequently transferred to lysosomes [62]. The MPR then recycles back to the surface. Previously, it has been shown that MPR can bind to Granzyme B (GrzB) and may play an important role in GrzB-mediated cell killing [63–65]. Recent data demonstrated that chemotherapy causes up-regulation of MPR on tumor cells in vitro and in vivo [60, 66], making the tumor cells permeable to GrzB produced by activating CTLs. This up-regulation was short lived. In mouse tumor models, chemotherapy-induced up-regulation of MPR was observed for only 3–4 days after the injection of drugs [66]. If adoptive T-cell therapy was administered to mice after the up-regulation of MPR disappeared, then the potentiated effect of combined therapy was not observed [66]. The timing of the administration of chemotherapy and immunotherapy has considerable importance. Available clinical data suggested that combination therapy was effective, when chemotherapy was given after the vaccines. Arlen et al. reported a prolonged time of progression in prostate cancer patients who received docetaxel after vaccine [67]. Garnett et al. [68] have shown that poxvaccine-induced immune responses, in the murine adenocarcinoma MC38 model, were enhanced when docetaxel was given after vaccine and diminished when the drug was given before, or concurrently, with vaccine. A recent study has successfully used CIS/gemcitabine after the administration of viral immunogene [69]. These data are consistent with the results of our experiments, where the potentiating effect of combined treatment was observed only when chemotherapy-induced up-regulation of MPR took place in the presence of CTLs. Recent study has shown that autophagy may play an important role in immunogenic signaling during chemotherapy. Post-chemotherapy release of ATP was higher in autophagy-competent tumor cells than in autophagy deficient ones. Autophagy deficient cells did not prime T cells in vivo or recruit CD4 and CD8 locally. Also, autophagy-competent cancers, but not the autophagy-deficient malignancies, attracted DCs and T cells to the tumor bed [70].

The blockade of MPR expression, with siRNA, reduced the GrzB uptake caused by different chemotherapeutic agents and substantially decreased the killing of tumor cells in the presence of CTLs [66]. Importantly, by making tumor cells susceptible to soluble GrzB, chemotherapy bypassed the requirement for perforin for CTL-mediated killing, thus making bystander tumor cells sensitive to CTLs without need for direct cell–cell contact and antigen recognition [60, 66].

Chemotherapy did not induce MPR synthesis or act as an inhibitor in its degradation. Instead, there was a redistribution of the receptors within the treated cells [66]. This redistribution was closely associated with autophagy, induced by chemotherapeutic agents. Inhibition of autophagy, with either 3-methyl adenine or by down-regulating atg5, abrogated chemotherapy-induced up-regulation of MPR on the tumor cell surface [66]. Many details of the mechanism by which autophagy may affect redistribution of the receptor remain unclear. We propose that autophagy, caused by chemotherapy, leads to redirecting MPR to the autophagosomes, either as clathrin-coated vesicles or as a result of the fusion of autophagosomes with endosomes (where MPR are usually located). In both cases, low pH in autophagosomes may result in the release of the MPR cargo, resulting in accumulation of empty receptors on the cells surface. This process may not only affect normal recycling of MPR, but also make these receptors capable of binding to GrzB. The internalization of GrzB protease then leads to apoptotic cell death.

## Conclusions

There is, now, substantial evidence suggesting that chemotherapy may potentiate the antitumor effect of immunotherapy. The mechanism of this phenomenon is not entirely clear. It is possible that chemotherapy, especially at low doses, may affect the host's immune system by augmenting an antigen-specific immune response via making tumor cells immunogeneic, by enhancing antigen processing and presentation, and by eliminating immune-suppressive MDSC and Tregs. Now, there is evidence that conventional chemotherapy may sensitize tumor cells to CTLs via the up-regulation of MPR, which could enhance the antitumor activity of CTLs by making tumor cells susceptible to soluble GrzB and thus expanding the cytotoxic effect of CTLs on neighboring cells. If this concept is confirmed in further studies, it may suggest a novel biomarker, useful in determining the likelihood of successful combination therapy, and also help to define the sequence and time of such therapy.

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