

Low-Dose Chemomodulation and Cancer Vaccines

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Recent advances in clinical and experimental oncology and immunology have markedly expanded our understanding of the role of the immune system response to precancerous cells, their malignization and expansion, as well as to tumor formation, growth and progression. These achievements resulted in development of improved and novel therapeutic modalities, which involve the treatment of cancer by modification of the host-tumor relationship. For instance, the main goal of antitumor immunotherapy is to provide either passive or active long-lasting immunity against malignancies by controlling the immune system responsiveness to tumors. Much of the success of anti-cancer immunotherapeutics has come from monoclonal antibodies delivering their biological activities either through immune-related effector functions, specific delivering of anticancer agents or by inhibiting dysregulated ligand-receptor interactions. However, despite their significant clinical success, antibodies have several well-known limitations, including expensive manufacturing, limited penetration into the tumor mass and some potential undesired systemic effects. Therefore, in addition to the therapeutic antibodies, immunomodulators (e.g., TLR ligands, thalidomide and its analogues, Lactoferrin), cytokines/chemokines/growth factors, cellular immunotherapy and vaccines have increasingly become thriving therapeutic agents for the treatment of solid and hematological malignancies in preclinical models, clinical trials and even clinical practice.

Although immunotherapy has emerged as an alternative option for the treatment of cancer patients in the last decades, the presented evidence indicates that both active and adoptive immunotherapeutic strategies are somewhat effective against small tumor burdens, but are generally insufficient to eliminate the disease in patients with advanced stage cancer, despite induction of tumor-specific immune responses [1]. Furthermore, while vaccine approaches have had some clinical achievement, most cancer vaccines fail to induce objective tumor shrinkage in patients. New modalities have centered on a group of molecules known as immune checkpoints, since their function is to contain or diminish potentially exuberant reactions. Antibody-based blocking of immune checkpoint molecules has emerged as a viable clinical approach that mediates tumor shrinkage in several cancer types. In addition to being part of the current treatment armamentarium for metastatic melanoma, immune checkpoint blockade is currently undergoing phase III testing in several cancer types [2]. Targeting co-inhibitory and co-stimulatory receptors with immunostimulatory antibodies has also shown clinical promise and its combined use with vaccines is a promising new approach of immunotherapy for cancer [1].

Advances in our understanding of the primary mechanisms of immune and cancerous cell interactions and the functioning of the tumor immunoenvironment have also provided the platform for combining cancer vaccines with chemotherapies neutralizing, to some extent, tumor-induced suppressive network and demonstrating clinical efficacy. A growing body of clinical data suggests that despite the great specificity that can be achieved with immunotherapy and the

potency of cytotoxic anticancer agents, neither of these two modalities by itself has been sufficient to eradicate the disease [3]. Therefore, the combination of chemotherapy and immunotherapy is no longer considered incompatible, because of the emerging insight that certain chemotherapy-based cancer treatments may activate the immune system against the tumor through several molecular and cellular mechanisms [4]. Indeed, certain chemotherapeutic agents have shown immunomodulatory activities, and several combined approaches have already been attempted [5].

Chemotherapeutics and immunotherapy may thus be synergistic and improve the clinical response in cancer. For instance, chemotherapy has been proven to enhance the efficacy of vaccination [6,7] and to favor the activity of adoptively transferred tumor-specific T cells or dendritic cells (DC) [8,9]. A potential efficacy of combining neo-adjuvant chemotherapy and immunotherapy with PBMC and IL-2 in the treatment of NSCLC patients has been recently reported [10]. Furthermore, when combined with CTLA-4 (a cytotoxic T lymphocyte antigen-4) blockade, ixabepilone, etoposide and gemcitabine elicited prolonged antitumor effects in several murine models with induction of a memory immune response [11]. Other data support the combinatorial use of immunomodulators, such as ipilimumab (anti-CTLA-4 antibody), with traditional chemotherapy regimens to improve SCLC patient outcomes and potentially sustain the effect from chemotherapeutic induction [12]. Synergy is mediated by diverse mechanisms, including preferential depletion of regulatory T cells, liberation of homeostatic or inflammatory cytokines and enhanced immunogenicity of chemotherapy-treated tumors. 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 [3]. 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 [1].

Given a growing enthusiasm to combining chemotherapeutic and immunomodulating agents for cancer treatment, it is important to notice that only a few cytotoxic drugs were proven to improve the therapeutic efficacy of cancer vaccines, while the majority of conventional chemotherapies are still immunosuppressive [5]. For

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instance, to examine the impact of clinically relevant doses of alkylating chemotherapeutics (temozolomide and cyclophosphamide) on cancer vaccines, Litterman et al. used controlled animal experiments that minimized the numerous complicating factors encountered in human patients [13]. The results clearly demonstrated that alkylating chemotherapy has a long-lasting antiproliferative effect on lymphocytes, and this effect leads to profound impairment of adaptive immune responses to cancer vaccines [14]. Furthermore, the immunomodulating effects of chemotherapeutic agents might be quite complex [15]. For instance, evaluation of two clinically used chemotherapeutic agents, gemcitabine and 5-fluorouracil, known to deplete protumorigenic myeloid-derived suppressor cells (MDSC), revealed that they may also activate the inflammasomes in MDSC, leading to production of interleukin-1 β (IL-1 β), which restricts anticancer immunity [16]. Next, when cyclophosphamide is administered in relatively lower doses than those routinely used in the clinical regimen, it can augment antitumor responses as it causes depletion of Treg cells [17]. In contrast, cyclophosphamide-induced MDSC accumulation was also reported by several authors [18-20], as well as low-dose cyclophosphamide-induced prevention of the development of antitumor immune responses [21]. Thus, selection of an appropriate clinically relevant combination of a chemotherapeutic drug and a cancer vaccine is far from been accepted and should be strictly based on the immunomodulating properties of a cytotoxic agent in relation to the mechanisms of the antitumor activity of a tested vaccine.

Finally, recent evaluations of potential immunomodulating activities of chemotherapeutic agents in ultra low noncytotoxic/noncytostatic doses resulted in introduction of a new direction of research – so-called, chemomodulation or chemoimmunomodulation, - when such activities have been proven [5]. In fact, several reports revealed that certain chemotherapeutic agents could up-regulate maturation and antigen-presenting activity of murine and human DC when used in ultra-low noncytotoxic concentrations *in vitro* [22-24]. Recent data demonstrated that ultra-low dose paclitaxel (Taxol) prevented tumor-induced polarization of conventional DC into immunosuppressive regulatory DC (regDC) *in vivo* and *in vitro* and up-regulated the antitumor potential of DC vaccines [25]. Similarly, Zhong et al. reported that a single administration of very low-dose paclitaxel synergized with DC vaccine in inhibiting lung cancer growth in mice [26]. Interestingly, paclitaxel in ultra-low concentrations was also able to support differentiation of MDSC into functionally active DC [27].

Analyzing the mechanisms of chemomodulation, Sevko et al. investigated the effect of paclitaxel applied in ultra-low, noncytotoxic doses on the efficiency of immunization of healthy mice with the peptide derived from tyrosinase related protein (TRP)-2 as a model melanoma antigen [28]. They found that administration of paclitaxel in combination with the peptide vaccination strongly increased the frequencies of TRP-2 specific T cells and was associated with a significant decrease in the levels of regulatory T cells and MDSC. Furthermore, in paclitaxel-treated mice, a significant augmentation of NK cell numbers and their ability to produce IFN- γ were also observed [28]. Using the ret transgenic murine melanoma model, which mimics human cutaneous melanoma, the same group has tested effects of ultralow noncytotoxic dose of paclitaxel on MDSC, chronic inflammatory mediators and T cell activities in the tumor microenvironment *in vivo* [29]. Administration of paclitaxel significantly decreased accumulation and immunosuppressive activities of tumor-infiltrating MDSC without

alterations of the bone marrow hematopoiesis. The production of mediators of chronic inflammation in the tumor milieu was also diminished. Importantly, reduced tumor burden and increased animal survival upon paclitaxel application was mediated by the restoration of CD8 T cell effector functions. This suggests that the ability of paclitaxel in a noncytotoxic dose to block the immunosuppressive potential of MDSC *in vivo* represents a new therapeutic strategy to down-regulate immunosuppression and chronic inflammation in the tumor microenvironment for enhancing the efficacy of concomitant anticancer therapies.

Altogether, a growing body of evidence supports a new concept that certain chemotherapeutic agents in ultra-low noncytotoxic doses may suppress tumor progression by targeting several immune cell populations in the tumor microenvironment, including DC and MDSC. New data also suggest that immunomodulating properties of certain chemotherapeutic agents in ultra low doses can be used to increase the therapeutic efficacy of cancer vaccines. However, more data are required in order to build a strong basis for developing novel clinical protocols testing the practicability and efficacy of augmentation of the antitumor properties of cancer vaccines combined with ultra-low noncytotoxic doses of chemotherapeutic agents.

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