Enhancing the efficacy of cancer vaccines in urologic oncology: new directions

Sergei Kusmartsev and Johannes Vieweg

Abstract | Immunotherapeutic interventions have long been utilized in urologic oncology for the treatment of metastatic renal cell or superficial transitional cell carcinoma. Most recently, the first active specific immunotherapeutic approach, a cancer vaccine, has passed the final phase of human testing and its approval by the FDA is pending. However, evidence suggests that the full protective and therapeutic potential of cancer vaccines has not yet been achieved. Through multiple mechanisms, tumors promote conditions in the tumor-bearing host that mitigate or even eliminate the vaccine-induced antitumor response. Restoration of the impaired immune function is, therefore, imperative for achieving optimum vaccine efficacy. Targeted pharmacological interventions are capable of overcoming tumor-mediated immunosuppression, and thereby enable cancer vaccination to reach its full therapeutic potential.

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Introduction

Over the past decade, a variety of immunotherapeutic approaches have been tested to stimulate the cellular and humoral arms of the immune system and to induce regression of malignant disease. Several investigational strategies are under consideration, including the application of cytokines and of adjuvant agents that modulate the cytokine response; therapeutic cancer vaccines designed to elicit cellular immune responses against tumor-associated antigens (TAAs); and monoclonal antibody drugs.[1] At present, only a few immunotherapeutic approaches have received approval by the FDA for treatment of urological malignancies, most notably the systemic administration of interleukin (IL) 2 against metastatic renal cell carcinoma (RCC) and the intravesical instillation of bacillus Calmette–Guérin or interferon α for superficial bladder cancer. Active specific immunotherapeutic approaches, in the form of cancer vaccines, have just begun to show therapeutic success in genitourinary and other malignancies,[2] and are, therefore, considered an attractive avenue for oncology research and drug development. In this Review, we first provide a concise update on the results of cancer vaccine trials in the field of urological oncology. We then highlight the problems presented by the phenomenon of tumor-mediated immunosuppression. Finally, we discuss novel translational interventions to counteract immunosuppressive pathways in the tumor microenvironment in order to enhance the immunological and clinical efficacy of cancer vaccines.

Department of Urology, Prostate Disease Center, University of Florida College of Medicine, Gainesville, FL, USA (**S. Kusmartsev**, **J. Vieweg**).

Correspondence: J. Vieweg, Department of Urology, University of Florida College of Medicine, Room N2-3 Health Science Center, P.O. Box 100247, Gainesville, FL 32610-0247, USA j.vieweg@urology.ufl.edu

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Cancer vaccines

Cancer vaccines are designed to stimulate expansion of the cellular arm of the immune system, especially T cells and natural killer cells. Cytotoxic and helper T lymphocytes are the main immune effector cells, which kill tumor cells with pinpoint accuracy via receptor-mediated interactions (Figure 1). Both cell types require activation by antigen-presenting cells, such as dendritic cells (DCs), to recognize and kill tumor cells in context with MHC self-antigens. Natural killer cells, by contrast, kill rather non-specifically and represent the first line of immunological defense against cancer and foreign pathogens (Box 1). Many vaccine approaches have shown high efficacy at triggering T-cell responses against TAAs in tumor-bearing animals—these approaches include vaccination with gene-modified tumor cells, antigen-loaded DCs, recombinant viral expression cassettes, and heat shock proteins.[3] All these approaches have now moved from the preclinical research arena into clinical trials, the results of which have been previously reviewed.[4] Although contemporary vaccine strategies are relatively effective at enhancing cellular immune responses against tumors in animals and humans, clinical responses and tumor regression have been observed in only a few clinical trials. Overall, rates of tumor regression have seldom exceeded 5–10%, and the discouragingly short duration of these responses (no more than a few months) has raised serious questions regarding the efficacy of this treatment approach.[3]

Many explanations have been offered for the limited capacity of cancer vaccines to induce tumor regression. First, therapeutic cancer vaccines must be initially studied in patients with advanced or metastatic disease, who typically exhibit a profoundly suppressed immune response. Second, the immunogenicity of the TAAs used in reported vaccine formulations is low, as most

TAAs represent self-antigens that are overexpressed or reactivated in cancer cells relative to the noncancerous cells from which they originated.[4–8] Finally, tumors can evade the immune system (including the immune responses triggered by vaccination) through the induction of immune tolerance or immune suppression,[9–13] as discussed in the later sections of this manuscript.

Key points

- Cancer vaccines are designed to stimulate an antitumor immune response via expansion of the cellular arm of the immune system, especially T cells or natural killer cells
- At present, the therapeutic effect of cancer vaccines in patients with advanced genitourinary cancers is limited
- In patients with cancer, tumors promote immunosuppression via multiple mechanisms including secretion of tumor-derived factors, mobilization of bone-marrow-derived suppressor myeloid cells and induction of T-regulatory cells
- An immunosuppressive microenvironment helps cancer cells to evade immune recognition and immune-mediated destruction
- Simultaneous targeting of tumor-induced immune suppression and administration of a cancer vaccine has great potential to boost the antitumor immune response and produce a more-powerful therapeutic effect than vaccination alone
- Clinical trials that are investigating combinatorial approaches that entail abrogation of tumor-induced immunosuppression followed by active immunotherapy are ongoing in many academic and industry programs

Recent results

Two clinical trials in patients with metastatic prostate cancer—one of gene-modified prostate tumor cells and the other of prostate acid phosphatase (PAP) antigen-loaded DCs—have recently received major attention. At the 2009 American Society for Clinical Oncology (ASCO) Genitourinary Cancer Symposium, Small and colleagues[14] reported the results of VITAL-2, a multicenter, randomized, phase III clinical trial designed to evaluate the safety and efficacy of GVAX® (Cell Genesys Inc., South San Francisco, CA, USA) immunotherapy in combination with docetaxel chemotherapy versus docetaxel chemotherapy and prednisone in patients with hormone-refractory prostate cancer.[14] GVAX® is a vaccine that consists of a mixture of allogeneic prostate tumor cells genetically engineered to secrete granulocyte–macrophage colony-stimulating factor (GM-CSF). This trial included only patients with taxane-naive, castration-resistant prostate cancer who had pain that required opioid analgesia; the primary end point was superiority in overall survival. The study was prematurely halted owing to an observed imbalance in death rates between the two treatment arms.

Overall, VITAL-2 enrolled 408 patients, 114 of whom died during the course of the trial: 67 in the GVAX® plus docetaxel combination treatment arm and 47 in the docetaxel–prednisone control arm. Analysis revealed no significant difference in the patients' baseline characteristics or toxic effects of treatment that could explain the imbalance in death rate. A survival advantage (14.1 months versus 12.2 months; hazard ratio 1.7, 95% CI 1.15–2.53) was seen in the control arm (docetaxel–prednisone) over the experimental arm. These results were surprising as earlier-phase studies had established a very favorable adverse-effect profile of the vaccine; adverse events mainly consisted of mild injection-site reactions and flu-like symptoms. While the cause of the poorer survival with the vaccine therapy is currently being investigated, this study highlights that caution must be exercised when conducting combinatorial drug trials, especially when the toxic effects and effector mechanisms of combined drug action have not been fully elucidated. Although several experimental studies have alluded to potential synergy between chemotherapy and vaccines, questions related to the mechanisms of action, the timing of administration, and the appropriate choice of agents are still unanswered.[11]

On a more-positive note, at the 2009 American Urological Association annual meeting, Schellhammer *et al.*[2] reported that sipuleucel-T (Provenge®; Dendreon Corporation, Seattle, WA, USA)—a vaccine consisting of autologous antigen-presenting cells activated *in vitro* by use of a recombinant fusion protein (PAP–GM-CSF)—increased the median survival of men with metastatic prostate cancer compared with a placebo control, without significant toxic effects.[2] In contrast to the prematurely terminated VITAL-2 trial, this randomized, placebo-controlled, phase III study (named IMPACT) enrolled only patients with asymptomatic or minimally symptomatic hormone-refractory prostate cancer ($n = 512$). The primary end point of this study was overall survival. The patients' demographic and baseline characteristics were similar in the experimental and control treatment arms. Patients who received sipuleucel-T had a 4.1-month improvement in their median survival (25.8 months versus 21.7 months) and a greater than threefold increase in survival at 36 months compared with those who received placebo. Interestingly, no significant delay was found in the time to objective disease progression and PSA response. These data are currently being reviewed by the FDA and their decision on this drug's approval is pending. No doubt the appropriate selection of patients, improvement in manufacturing processes, the choice of adjuvant strategies, and the development of biomarkers to measure treatment success will all be critical to upcoming trials with this and other vaccine approaches. As these trials were phase III registration trials with survival as the end point, immune response analysis was not reported. However, earlier phase I–II trials demonstrated that sipuleucel-T can induce PAP-specific T-cell responses, while GVAX® induced an eosinophilic infiltrate at the injection site.

Tumor-mediated immunosuppression

Cancer vaccines are designed to activate and mobilize the host's adaptive immune response against tumors in

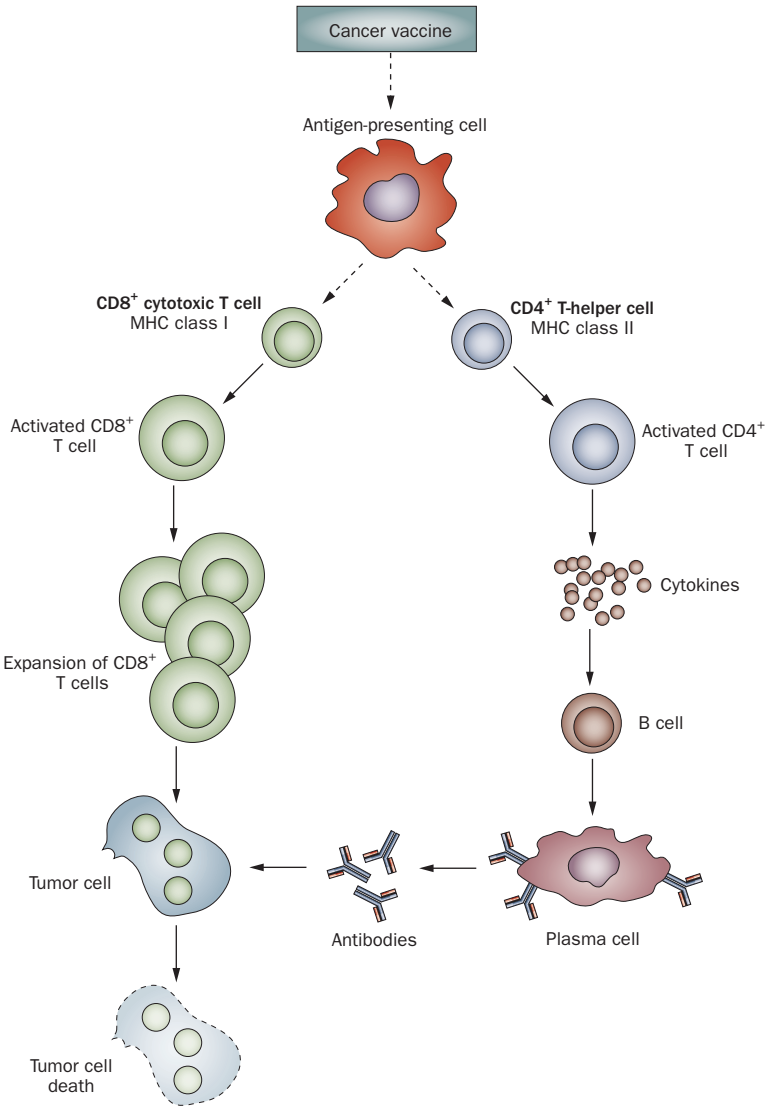

Figure 1 | The T-cell response against cancer. Cancer vaccines stimulate a T-cell response via activation by antigen-presenting cells, including dendritic cells. Cytotoxic $CD8^+$ T cells recognize and kill the tumor cell directly. $CD4^+$ T-helper cells release cytokines, which stimulate the activity of B cells, resulting in the production of antibodies against the tumor-associated antigens.

a targeted, antigen-specific fashion. Despite considerable progress in the identification of TAAs and in the development of effective vaccination strategies, the clinical results of therapeutic cancer vaccination have been disappointing thus far. One of the reasons for the lack of success is that virtually all antitumor vaccines were initially studied in patients with bulky or metastatic disease. However, patients with advanced malignancy have a compromised immune system and exhibit alterations in the proliferative and cytotoxic capacities of their T cells and natural killer cells, and in host cytokine expression and production.[9–11,15,16]

Ample evidence indicates that distinct compartments of the tumor microenvironment directly modulate the immune system via secretion of immunosuppressive factors such as TNF, TGF-β1, vascular endothelial growth factor (VEGF), G-CSF, and GM-CSF (Figure 2). These gene products mediate the conversion of tumor-infiltrating and peripheral myeloid cells—bone-marrow-derived precursor cells that give rise to various hematopoietic cells such as leukocytes, monocytes, and DCs—into immunosuppressive, myeloid-derived suppressor cells (MDSCs), tumor-associated macrophages, or T-helper-2-biased, tolerogenic DCs.[16,17] MDSCs express the transmembrane glycoprotein CD11b and are key mediators of immunosuppression in the tumor-bearing host and of tumor growth via the stimulation of angiogenesis and tumor progression. MDSCs can be divided into two major categories, namely $CD33^+CD11b^+$ myelomonocytic cells[18–20] and $CD15^+CD11b^+CD14^-$ granulocyte-type cells.[20–22] Both these cell types are profoundly immunosuppressive and represent independent lineages that arise from myeloid precursor cells. A typical hallmark of MDSCs is the overexpression of arginase 1, a key enzyme in the inhibition of T-cell responses.[23,24] Moreover, oxidative stress and secretion of nitric oxide (NO) by MDSCs contribute to the hostile, immunosuppressive tumor microenvironment by attenuating T-cell function.[25–28] MDSCs are frequently detected in the peripheral blood of patients with metastatic RCC[18,19,21,29] or bladder carcinoma (E. Eruslanov, personal communication). Moreover, MDSC frequencies seem to correlate with the stage of the disease, and potentially provide an important biomarker of prognosis.

Studies have shown that MDSCs can trigger expansion of another important immunosuppressive cellular subset, termed T-regulatory cells (T_{REG}).[30] T_{REG} represent an important, highly specialized subpopulation of $CD4^+$ T-helper cells that suppress activation of other T cells, especially of those specific against self-antigens.[31] Interest in T_{REG} biology has been heightened by recent studies that demonstrate enhanced cancer-vaccine-mediated immunity after T_{REG} elimination.[32] In humans, T_{REG} are characterized by expression of the high-affinity CD25 α-chain of the IL-2 receptor (IL-2R) and the transcription factor forkhead box P3 (FOXP3). As with MDSCs, significantly elevated T_{REG} levels are found in the peripheral blood of patients with cancer, and increased T_{REG} frequencies are associated with reduced overall survival.[32,33]

As MDSCs and T_{REG} are major contributors to cancer-mediated immunosuppression, considerable interest exists in further investigating these cellular subsets and in developing pharmacological interventions to reduce their immunosuppressive properties and, thereby, stimulate immune responses against cancer. In the following sections, we will delineate the molecular pathways associated with MDSC-mediated and T_{REG}-mediated immunosuppression and provide novel insights into translational strategies that might counteract immunosuppressive networks in the tumor microenvironment in urological cancer (Table 1).

Targeting of MDSCs

The importance of immunosuppressive pathways in tumors is well accepted by basic science and clinical investigators. As a result, the concept of favorably altering the tumor microenvironment to enable immune effector cells to interact with their tumor targets has become an exciting new direction in translational immunological research. Owing to the highly complex organization of the tumor microenvironment, a variety of approaches that target specific pathways are presently being considered in order to achieve favorable tumor tissue conditioning for therapy and to reverse the negative effect of tumors on immune function.

The receptor tyrosine kinase pathway

Receptor tyrosine kinase inhibitors represent a rapidly growing family of drugs with demonstrated clinical activity against malignancy, especially RCC. These agents target critical growth pathways that modulate tumor progression and metastatic potential.[34] Moreover, some of these agents induce immunostimulatory effects by downregulating the secretion of immunosuppressive factors in the tumor microenvironment.[35,36] For example, the multikinase inhibitor sunitinib shows both antitumor and immunomodulatory activity after oral administration. In patients with metastatic RCC, sunitinib significantly reduced MDSC and T_{REG} numbers in the peripheral blood.[35,36] Moreover, improved T-cell responses and abrogation of MDSC-mediated immunosuppression were achieved via reduction of levels of immunosuppressive cytokines and transcription factors and enhancement of interferon γ secretion by T cells.[37] Importantly, tumors of sunitinib-treated mice exhibited remarkable infiltration of cytotoxic $CD8^+$ T cells and $CD4^+$ T-helper cells, which suggested drug-induced activation and successful homing of T cells into tumors.

The antitumor and immune-enhancing effects of sunitinib might be mediated via a common mechanism, namely inhibition of signal transducer and activator of transcription 3 (STAT3).[38] STAT3 is not only constitutively activated in various cancer cells, but also in MDSCs, which thereby inhibits differentiation of DCs and promotes tumor immune evasion (Figure 3).[39] Cumulatively, these data suggest that sunitinib, and maybe other receptor tyrosine kinase inhibitors, can be used effectively to reverse tumor-induced immunosuppression. Thus, the clinical evaluation of combinatorial regimens that study potential synergism between sunitinib and immunological approaches is ongoing in many academic and industry settings.

PGE_2 metabolism

Prostaglandin E_2 (PGE_2) is a key molecule that prevents the generation of antitumor immune responses by inhibiting maturation and function of DCs.[40,41] Moreover, intratumoral PGE_2 promotes tumor growth by activating signaling pathways that regulate cell proliferation, migration, apoptosis, and angiogenesis.[40,41] Intratumoral PGE_2 levels are physiologically regulated through NAD^+-linked 15-hydroxyprostaglandin dehydrogenase (15-PGDH), an enzyme that facilitates degradation of PGE_2. Expression and catabolic activity of 15-PGDH is frequently decreased in cancer tissues,[42] which causes profound immunosuppression via increased intratumoral levels of PGE_2. Loss of 15-PGDH expression correlates with tumor progression in colorectal, lung, and transitional cell bladder cancers.[40] We have shown that targeted overexpression of *HPGD* (the gene encoding 15-PGDH) at the tumor site can significantly reduce PGE_2 levels in the tumor microenvironment and promote a therapeutic antitumor response in animals that carry prostate or colon carcinomas.[43] Thus, forced expression or pharmacological stimulation of 15-PGDH in the tumor microenvironment with recombinant expression vectors or other gene delivery systems might represent an effective approach to reduce immunosuppressive intratumoral PGE_2 levels and to promote stimulation of effective antitumor immune responses, both innate and vaccine-induced.

A second major approach by which intratumoral PGE_2 synthesis can be modulated is via inhibition of cyclo-oxygenase 2 (COX-2).[44] COX enzymes convert arachidonic acid to prostaglandins, including PGE_2, and thromboxane. Recent clinical research suggests that aspirin and other NSAIDs, which are well-characterized inhibitors of COX-2, can reduce the incidence of certain human malignancies, including colorectal and prostate cancers.[45,46] Selective COX-2 inhibition facilitates cytotoxic $CD8^+$ T-lymphocyte infiltration of tumors, elevates systemic IL-12 and interferon γ levels, and reduces levels of IL-10[47–50] and MDSCs in a tumor-bearing mouse host.[51,52] In addition, adjunctive COX-2 inhibitors increase the potency of cancer vaccines and prolong survival in experimental animals.[53,54] Moreover, the clinically available compound NO-aspirin (nitric-oxide-releasing aspirin) interferes with the T-cell inhibitory activity of

Box 1 | Glossary of the immune system

- Cytotoxic T cells: killer T lymphocytes capable of killing tumors through receptor-mediated interactions
- T-helper cells: a subgroup of lymphocytes that provide critical help for cytotoxic T lymphocytes to recognize and kill tumor cells
- T-regulatory cells (T_{REG}): a specialized subpopulation of T lymphocytes that act to suppress activation of the immune system
- Dendritic cells (DCs): the most potent antigen-presenting cell type of the immune system
- Natural killer (NK) cells: a type of cytotoxic lymphocyte that has a major role in the rejection and killing of tumors
- Myeloid cells: hematopoietic precursor cell that gives rise to lymphocytes, monocytes, and antigen-presenting cells
- Myeloid-derived suppressor cells (MDSCs): a cell type that exerts an immunosuppressive effect, presumably through release of radical oxygen species and nitric oxide
- Tumor-associated microphages (TAMs): a cellular subset derived from myeloid cells with immunosuppressive activity

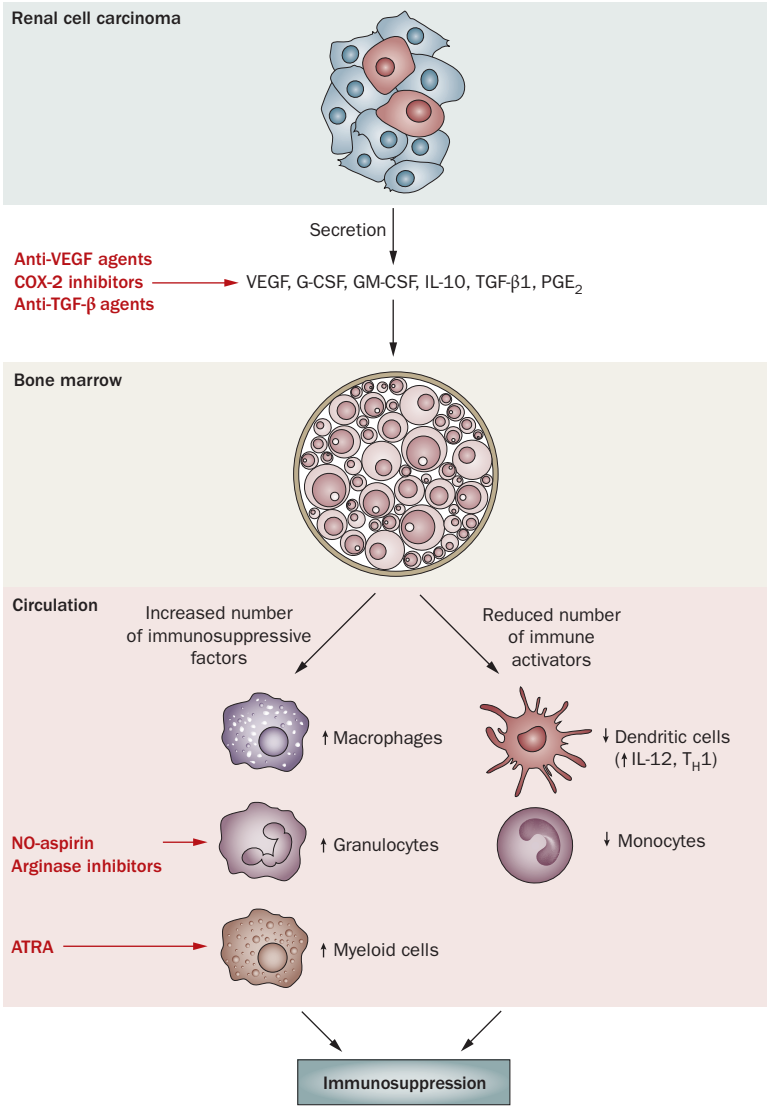

Figure 2 | Elevated numbers of immunosuppressive myeloid cells in the peripheral blood of patients with cancer. Tumor cells, for example renal cell carcinoma, secrete a variety of bioactive products that stimulate mobilization of immature myeloid cells from bone marrow into the peripheral blood. These cells are profoundly immunosuppressive and support tumor growth via inhibition of adaptive immune responses. By contrast, the proportion of T_H1-biased dendritic cells and their immediate precursors are reduced in patients with cancer. Both the secreted products and myeloid cells are candidates for targeted therapies to enhance the efficacy of cancer vaccines (red text). Finally, many tumor-recruited myeloid cells migrate into tumor tissues where they support tumor growth via local immunosuppression and stimulation of neoangiogenesis (see Figure 3). Abbreviations: ATRA, all-*trans* retinoic acid; COX-2, cyclo-oxygenase 2; G-CSF, granulocyte colony-stimulating factor; GM-CSF, granulocyte–macrophage colony-stimulating factor; IL, interleukin; NO-aspirin, nitric-oxide-releasing aspirin; PGE_2, prostaglandin E_2; TGF-β1, transforming growth factor β1; T_H1, T-helper lymphocyte type 1; VEGF, vascular endothelial growth factor.

MDSCs.[55] NO-aspirin possesses no direct antitumor activity, but it can normalize the immune status of tumor-bearing animals by increasing the number and function of TAA-specific T lymphocytes and by enhancing the efficacy of the vaccine-induced immune response.

These preclinical data provide the scientific rationale for two ongoing clinical trials that are exploring the synergistic effects of cancer vaccines and NO-aspirin in patients with advanced neoplastic diseases.[19,56] Despite their favorable effects on the immune system, prolonged use of high-dose COX-2-selective inhibitors is associated with unacceptable cardiovascular adverse effects.[57,58] These effects must be carefully considered in the design, planning, and execution of clinical trials that investigate potential synergy between COX-2 inhibitors and immunological treatments for cancer.

Arginine metabolism

Increased metabolic activity of L-arginine in MDSCs and tumor-associated macrophages triggers impairment of T-lymphocyte responses to tumor antigens.[59] Essential components of this T-lymphocyte suppression pathway include two enzymes that compete for L-arginine as a substrate: arginase and NO synthase. Arginase is, therefore, implicated in the regulation of synthesis of both NO and polyamines, both of which are detrimental to T-cell function. Cancers exhibit an altered arginine metabolism because expression of arginase is upregulated in the tumor microenvironment. Increased arginase activity reduces access to free arginine and blocks the proliferation of T-effector cells as a result of arginine starvation.[60] Both arginase type 1 and 2 have been implicated in tumor-induced immune dysfunction in metastatic RCC[22] and prostate cancer.[25] Importantly, expression of arginase in myeloid cells is inducible and can be regulated by type II cytokines, such as IL-4 and IL-13, and by PGE_2.[61] Preclinical studies have shown that inhibition of arginase activity significantly delays tumor growth in mice.[61]

These results indicate that arginase modulates the L-arginine metabolic pathway to regulate both T-cell function and tumor cell growth. Blockade of arginase type 1 or 2 in a clinical setting might aid in the restoration of immune function by increasing L-arginine availability for T-cell use and might lead to a decrease in tumor cell growth. Targeting the relationship between arginase and the immune system might offer therapeutic approaches for patients with advanced malignancies.

IDO and tryptophan metabolism

Indoleamine 2,3-dioxygenase (IDO) is an immunosuppressive molecule that regulates T-cell proliferation and differentiation[62] by reducing access to free tryptophan, an amino acid essential for T-cell proliferation.[63,64] IDO is expressed by immunosuppressive DCs, which can be found in tumor tissues and draining lymph nodes of patients with advanced malignancy.[63] As IDO has a major role in the escape of malignant cells from immunological attack, blocking IDO-mediated pathways should improve antitumor responses and potentially lead to tumor regression. Considerable efforts are underway to develop IDO inhibitors that mitigate pathological immunosuppression for clinical trials.[65] In this regard, the competitive IDO inhibitor 1-methyltryptophan (1MT) significantly delays

Table 1 | Pharmacological interventions to overcome tumor-induced immune suppression

Target	Agent	Tumor type	Stage of development
RTKs	Sunitinib[22,35–37]	RCC, colon carcinoma	Clinical studies;[35,36] animal studies[37,38]
PGE_2 metabolism	COX-2 inhibitors,[61] 15-PGDH[50,52,95]	Lung adenocarcinoma, colon carcinoma	Animal studies[43,53,54]
Arginine metabolism	Nor-NOHA, L-norvaline[23,24,95]	Prostate adenocarcinoma, mammary carcinoma, lung cancer	Clinical study;[25] animal studies[61,95]
Tryptophan metabolism	1-methyltryptophan[62]	Breast cancer, melanoma	Animal study[66]
BMDC recruitment	CXCR4 antagonists, anti-VEGF agents[25,52,81]	RCC, melanoma, other	Clinical studies;[83,84] animal study[85]
T_{REG}	Denileukin diftitox,[32] cyclophosphamide[91]	RCC, melanoma, other	Clinical study;[32] animal study[94]
MDSC	ATRA[64,86]	RCC, mammary carcinoma	Animal study;[86] clinical trial[56]
MDSC	PDE5 inhibitors[56]	Colon carcinoma, mammary carcinoma	Animal study[89]
MDSC	Gemcitabine[89]	Lung cancer	Animal study[87]
MDSC	NSAIDs[87]	Colon carcinoma	Animal study[55]

Abbreviations: 15-PGDH, 15-hydroxyprostaglandin dehydrogenase; ATRA, all-*trans* retinoic acid; BMDC, bone-marrow-derived cell; COX-2, cyclo-oxygenase 2; CXCR4, C-X-C chemokine receptor type 4; MDSC, myeloid-derived suppressor cell; Nor-NOHA, Nω-hydroxy-nor-L-arginine; PDE5, phosphodiesterase type 5; PGE_2, prostaglandin E_2; RCC, renal cell carcinoma; RTK, receptor tyrosine kinase; T_{REG}, T-regulatory cells; VEGF, vascular endothelial growth factor.

tumor growth in mouse models.[66] Moreover, a synergistic therapeutic effect of 1MT in combination with immunotherapy, chemotherapy, or radiation therapy has recently been demonstrated.[65] Aside from 1MT-based IDO inhibition, gene silencing strategies based on small interfering RNAs and small-molecule inhibitors are currently being developed with the aim of reversing immunosuppression and halting tumor progression by blocking the IDO suppression pathway.[67]

Recruitment of bone-marrow-derived cells

Tumor-recruited bone-marrow-derived cells (BMDCs) mediate tumor-induced immune suppression, promote tumor vascularization, and stimulate tumor metastasis.[68–71] BMDCs represent a mixed cell population that includes hematopoietic progenitor cells, myeloid cells, and precursors of endothelial and stromal cells. Seemingly, tumor growth and metastatic development can be prevented by reducing the trafficking of BMDCs into tumor sites (Figure 3).[25] Several molecules and mechanisms can modulate tumor-mediated recruitment of BMDCs, including VEGF receptors and chemokine CXCL12–CXCR4-mediated pathways.[17,72–77] Overexpression of VEGF promotes mobilization of $VEGFR1^+CXCR4^+$ BMDCs into the peripheral blood, and thus promotes immunosuppression. Tumor-derived VEGF also inhibits differentiation of DCs and promotes accumulation of MDSCs in the tumor-bearing host.[78–80] Importantly, a considerable interplay seems to exist between the CXCR4 and VEGFR1 pathways as both receptors are highly upregulated under conditions of hypoxia or oxidative stress.[26,81] Targeting the VEGF–VEGFR and stromal cell-derived factor 1 (SDF-1)–CXCR4 axes, therefore, is a promising approach for immunomodulatory cancer therapy. Pharmacological inhibition of VEGF is now widely applied in the clinical setting as VEGF is overexpressed by many tumors, including RCC and colorectal carcinoma.[82] Example drugs include the monoclonal antibody bevacizumab and the fusion protein aflibercept, both of which have shown antitumor activity in clinical trials.[83] Synergistic activity has been reported in preclinical studies when cancer vaccines are combined with VEGF blockade.[84] Also, pharmacological inhibition of the SDF-1–CXCR4 axis promotes antitumor immunity by facilitating T-cell attraction to and lymphocyte infiltration of tumors.[85]

MDSC differentiation

Studies have demonstrated that the application of growth and differentiation factors facilitates differentiation of MDSCs into immunostimulatory DCs and other antigen-presenting cells.[19] These experiments further suggest that overcoming tumor-mediated arrest of differentiation in myeloid cells and stimulating potent immune responses in the context of cancer vaccination are possible. Accordingly, we and others have shown that all-*trans* retinoic acid (ATRA, a retinoid acid derivate with antineoplastic activity) can promote differentiation of MDSCs into DCs *in vitro* and *in vivo*.[19,56,86] Moreover, ATRA-mediated depletion of MDSCs in the peripheral blood of patients with RCC resulted in improved $CD4^+$ and $CD8^+$ T-cell responses and significantly enhanced the efficacy of cancer vaccination.[19,56] These results have prompted several ongoing clinical trials that include ATRA as part of a multimodal approach against RCC and other carcinomas.

Similarly, gemcitabine, a chemotherapeutic agent effective against various forms of solid tumor, reduces levels of $Gr\text{-}1^+CD11b^+$ MDSCs in mice that bear large tumors of different origins.[87] Depletion of MDSCs was

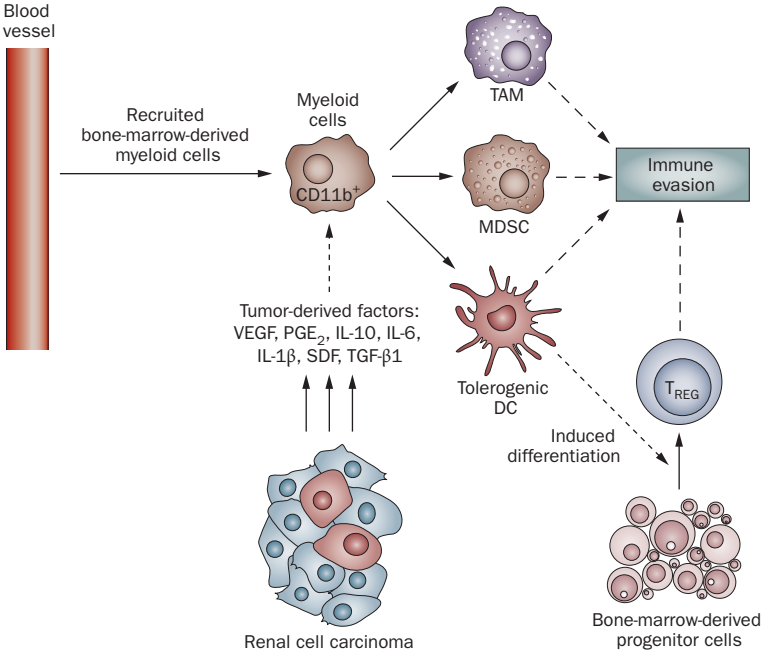

Figure 3 | Tumor-infiltrated myeloid cells contribute to the immunosuppressive network in tumors. Tumors, such as renal cell carcinoma, are capable of recruiting bone-marrow-derived myeloid cells, which differentiate into immunosuppressive, M2-polarized, arginase-expressing TAM, MDSC or T-helper lymphocyte type 2 oriented, tolerogenic DC. TAM inhibit local T-cell responses via arginase and iNOS-dependent peroxynitrite production. Intratumoral tolerogenic DC inhibit T-cell responses via IDO-dependent mechanisms or through induction of T_{REG}. Abbreviations: DC, dendritic cells; IDO, indoleamine 2,3-dioxygenase; IL, interleukin; iNOS, inducible nitric oxide synthase; MDSC, myeloid-derived suppressor cells; PGE_2, prostaglandin E_2; SDF, stromal-cell-derived factor; TAM, tumor-associated macrophages; TGF-β1, transforming growth factor β1; T_{REG}, T-regulatory cells; VEGF, vascular endothelial growth factor.

accompanied by an increase in the antitumor activity of $CD8^+$ cytotoxic T cells and by markedly enhanced antitumor efficacy in animals inoculated with tumor cells, which suggested that gemcitabine might be a realistic strategy for reducing levels of MDSCs in humans in conjunction with either active or passive immunotherapeutic approaches. While the exact mechanism of immune enhancement for gemcitabine is currently under investigation, its activity might be explained by increased chemosensitivity of MDSCs or a differentiation-promoting action of the drug.

The phosphodiesterase type 5 pathway

Phosphodiesterase type 5 (PDE5) inhibitors are a drug class currently in clinical use for various nonmalignant disorders, including pulmonary hypertension and erectile dysfunction.[88] Interestingly, sildenafil downregulates expression of arginase 1 and NO synthase 2, and thereby limits recruitment of immunosuppressive $Gr\text{-}1^+CD11b^+$ MDSCs by the enlarging tumor. Sildenafil-mediated PDE5 inhibition has no direct effect on tumors, but the drug reverses tumor-induced immunosuppression and is associated with a measurable antitumor immune response in mice.[89] Moreover, sildenafil enhances intratumoral T-cell infiltration and activation, reduces tumor outgrowth, and improves the antitumor efficacy of adoptive T-cell therapy. As tumor-derived factors that mediate MDSC-dependent immunosuppression in mice are also active in humans, these data suggest a potentially novel use of PDE5 inhibitors as adjuncts to tumor-specific immunotherapy.

Targeting of T_{REG}

The ability of tumors to evade destruction by immune effector cells is an important characteristic that enables their uncontrolled growth and survival. Novel insights suggest that tumor immune evasion can be controlled by the modulation of cellular subsets that control immune responses to self-antigens.[90] Among these approaches are therapies directed against $CD4^+$ T_{REG}, a cellular subset capable of suppressing $CD4^+$ and $CD8^+$ effector T cells through the secretion of suppressive cytokines, IL-2 consumption, and cytolysis. T_{REG} physiologically dampen the function of antigen-presenting cells by decreasing costimulation or antigen presentation, particularly against self-antigens.[31] T_{REG} are, therefore, important regulators that prevent autoimmune responses and recognition of self-antigens via direct cell-to-cell interactions. Enhanced understanding of the immune-attenuating mechanisms of T_{REG} might offer insight into how T_{REG} function can be manipulated in context with other immunomodulatory strategies.

On the basis of the fundamental role of T_{REG} in suppression of antitumor immunity, depleting or functionally inactivating this cellular subset in patients with cancer has been proposed as a strategy to boost the efficacy of cancer immunotherapy.[91]

Targeting of CD25

Studies have shown that T_{REG} can be efficiently depleted by monoclonal antibodies that target CD25, the α-chain of IL-2R, and that such treatment can induce the rejection of transplantable tumors.[92] CD25 is present on the cell surface of a variety of hematopoietic cells but is overexpressed by T_{REG}, which forms the basis for novel interventions by use of compounds with high selectivity for CD25.

Denileukin diftitox is a fusion protein consisting of full-length IL-2 fused to the enzymatically active and translocating domains of diphtheria toxin. This protein is nontoxic, but it inhibits protein synthesis after internalization into the cytosol of CD25-expressing cells via intracellular release of diphtheria toxin. Denileukin diftitox is capable of selectively killing IL-2R-expressing cells, such as activated T cells and some tumor cells that express CD25.[93] Malignant cells that express one or more subunits of IL-2R can be found in certain leukemias and lymphomas, some solid tumors of the head and neck, non-small-cell lung cancer, RCC, and sarcoma.

The exquisite selectivity for the CD25 domain of IL-2R and its thorough characterization in prior clinical trials make denileukin diftitox a suitable reagent for *in vivo*

depletion of $CD25^+$ T_{REG}. A combinatorial regimen consisting of denileukin diftitox followed by DC-based vaccination selectively eliminates T_{REG} with high expression of CD25 from the peripheral blood mononuclear cells of patients with metastatic RCC without toxic effects on other cellular subsets with intermediate or low expression of CD25.[32] Importantly, anti-CD25-mediated T_{REG} depletion resulted in enhanced stimulation of proliferative and cytotoxic T-cell responses *in vitro*, but only when denileukin diftitox was omitted during the T-cell-priming phase. In patients with metastatic RCC, denileukin-diftitox-mediated elimination of T_{REG} followed by vaccination with tumor-RNA-transfected DCs significantly improved tumor-specific T-cell responses compared with vaccination alone.[32] These data suggest the feasibility of transiently purging T_{REG} from patients with cancer to enhance a vaccine-mediated antitumor effect.

Nonspecific T_{REG} depletion

Animal experiments have shown that treatment with cyclophosphamide, a cytotoxic agent with a dual effect on the immune system, can decrease T_{REG} numbers in a dose-dependent fashion.[94] Aside from nonspecific cytotoxic activity, additional mechanisms that might explain cyclophosphamide-mediated T_{REG} elimination include the direct inhibition of T_{REG} function, enhancement of tumor antigen presentation, and amplification of T-helper-1-biased $CD4^+$ T-cell responses. Unfortunately, cyclophosphamide-mediated T_{REG} depletion seems to be rather nonspecific, as other $CD4^+$ T-cell populations, such as naive $CD4^+$ T cells ($CD4^+CD25^-$) or memory and effector $CD4^+$ T cells ($CD4^+$ and intermediate expression of CD25), are depleted following systemic cyclophosphamide therapy. Nevertheless, clinical trials that seek to deplete T_{REG} with low-dose cyclophosphamide followed by active immunotherapy are currently ongoing in several institutions.

Conclusions and future perspectives

Novel insights into tumor immunology and biology have accelerated efforts to bring new cancer therapies into clinical practice. Immune-based therapies, such as cancer vaccines, have demonstrated efficacy at inducing immunological antitumor responses in patients with cancer. However, vaccine-induced clinical responses are sporadic and generally of only short duration. As a possible explanation, tumor-mediated immune suppression represents a major obstacle to the stimulation of T-cell responses for clinical effect. Thus, novel strategies to abrogate tumor-mediated immunosuppression and restore immunocompetence in these patients are mandatory. Studies show that by counteracting local or peripheral immunosuppression, the potential for a therapeutic response to cancer vaccines is markedly enhanced. A thorough understanding of the mechanisms that mediate immune evasion is, therefore, necessary to define and develop new molecular targets for therapy. Targeting these molecules might augment vaccine-induced immune responses and improve clinical outcomes in patients with cancer.

Cancer is a complex disease, and successful vaccine therapy will require a multimodal or combinatorial treatment approach. Critical elements of such a concerted therapeutic strategy include not only improved vaccine formulations, but also strategies to reverse immunosuppressive pathways in the tumor microenvironment. Future clinical trials will provide better insight into the optimum combination of agents to reverse tumor-mediated immunosuppression and induce T-cell responses with clinical impact.

Review criteria

In addition to articles known to the authors, the full PubMed and MEDLINE databases and proceedings of the American Society for Clinical Oncology annual meetings were searched for published papers and abstracts using the following search terms: "cancer vaccines", "[vaccines] AND [urologic cancers]", "[prostate cancer] AND [vaccines]", "tumor microenvironment", "tumor-induced immunosuppression", "myeloid-derived suppressor cells", and "regulatory T cells". Papers were selected for inclusion on the basis of level of evidence and relevance to the topic. Only articles published in English have been cited in this Review.

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