Immune modulation of the tumor microenvironment for enhancing cancer immunotherapy

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Keywords: immunotherapy, tumor microenvironment, regulatory T cells, macrophages, immunosuppression

There is much promise in the use of immunotherapy for the treatment of cancer. Approaches such as those using antibodies or adoptive cell transfer can mediate complete tumor regression in a proportion of patients. However, the tumor microenvironment can inhibit immune responses leading to ineffective or suboptimal responses of tumors to immunotherapy in the majority of cases. As our knowledge of the tumor microenvironment increases, many strategies are emerging for changing the immunosuppressive nature of the tumor toward a microenvironment able to support immunity. These strategies aim to enhance the ability of immunotherapies to initiate effective immune responses able to destroy tumors. In this article, we review approaches that use immunomodulators specifically to modify the tumor microenvironment, and their use in combination with other immune-based strategies for cancer therapy.

Introduction

Immunotherapy holds much promise for the treatment of cancer. A wide variety of approaches have been implemented in order to stimulate a range of immune activities including innate and adaptive components. Strategies include the use of immunomodulatory antibodies, vaccines and adoptive cell transfer. Notable clinical successes include the use of the immune check-point inhibitor, ipilimumab[1] for melanoma, and rituximab targeting CD20 for lymphoma.[2] Adoptive immunotherapy, involving transfer of ex vivo activated autologous T cells, is also showing promise for the treatment of melanoma.[3]

However, most immunotherapeutic approaches on their own are of limited value against the majority of malignancies.

Reasons for this limited success include immune regulation mediated by cancer cells and leukocyte populations through a variety of cell-expressed and secreted molecules. In many cases, immune regulation occurs locally within the tumor, leading to an ineffectual or suppressed antitumor response.

Tumors are not just a mass of proliferating genetically abnormal cells, but they are now well defined as a heterogeneous and structurally complex tissue. Malignant tumor cells can recruit a variety of cell types, including fibroblasts, immune inflammatory cells, and endothelial cells, through production and secretion of stimulatory growth factors and cytokines.[4] This assortment of cells and molecules together comprises the tumor microenvironment.

Antitumor immunity within the tumor microenvironment can be suppressed by a variety of tumor infiltrating leukocytes, including regulatory T cells (Treg), myeloid-derived suppressor cells (MDSC) and alternatively activated (type 2) macrophages (M2).[5-7] Mechanisms employed by these cell types to suppress effective immunity include secretion of cytokines such as IL-10 and TGFβ, and expression of inhibitory receptors such as CTLA-4 and PD-L1. Secretion of amino acid-depleting enzymes including arginase and IDO by these cell types in the microenvironment can also negatively impact on tumor immunity.

In addition to these effects mediated by infiltrating cells, tumor cells themselves can actively inhibit immunity through a number of mechanisms. Malignant cells can block T cell function through secretion of soluble forms of ligands for effector molecules, as reported for shed ligands of NKG2D; MICA and MICB.[8] Additionally, cytokines released by tumor cells, such as VEGF and TGFβ can inhibit T cell recognition and destruction of malignant cells.[9] IL-10 as well, can skew T cell responses toward a type 2 immune response that is less effective against tumor cells.[10] Other secreted factors such as galectins can also impede T cell activity and survival.[11]

Many of these regulatory mechanisms can occur concurrently within the tumor microenvironment resulting in multiple redundant levels of immune suppression, which reduces the effectiveness of immunotherapy. Not surprisingly then, the tumor microenvironment can impede immunotherapy, and approaches to specifically reduce immune suppression within the tumor microenvironment are gaining momentum as a companion to

additional immunotherapy. This review focuses on immune-based strategies to change the microenvironment to enable the effectiveness of immunotherapy, with discussion largely restricted to studies that demonstrate changes to the tumor microenvironment and synergy between that and additional immunotherapy.

Check Point Inhibitors

Immune inhibitory receptors can be expressed on, or secreted, by tumor cells and stromal components and constitute an important part of the tumor microenvironment. A variety of molecules, often referred to as immune checkpoints, including PD-1 and TIM3 can mediate immune inhibition through their respective inhibitory ligands, PD-L1 and galectin 9, expressed by tumor cells.[12] CTLA-4, which can be expressed by antigen presenting cells, is an inhibitory competitor for CD80 and CD86 costimulation of T cells through CD28, which can effectively inhibit T cell activation and expansion. Blockade of CTLA-4 interactions can itself enable endogenous immunity against tumors, and promising results have been observed in clinical and preclinical settings.[1,13,14] However, targeting immune checkpoints to reduce an immunosuppressive microenvironment within tumors in combination with other immunotherapies can result in dramatically improved antitumor responses.

Programmed Cell Death-1 (PD-1) is expressed on activated T cells, B cells and myeloid cells, and can induce inhibition and apoptosis of T cells following ligation by programmed death ligands-1 or -2, the former of which can be expressed on tumor cells. The PD-1 pathway performs a crucial role in the normal regulation of immunity, but the utilization of this pathway by tumors can inhibit immune control of malignancy. Agents in use for blocking the PD-1 pathway include neutralizing antibodies and soluble PD-1 ligands (**Fig. 1**).

Immune therapies used in combination with PD-1 include adoptive transfer of tumor-reactive T cells, where enhanced tumor localization of T cells was observed together with increased inhibition of tumor growth.[15] While PD-1 blockade can augment passive transfer of immunity, it can also be used to enhance endogenous antitumor immune responses as seen in studies that combine it with anti-CD137 or tumor cell vaccines expressing Flt3L or GM-CSF, which could prolong survival of mice[16] or even eradicate tumors in some cases.[17,18]

Therapies targeting PD-1 or CTLA-4 are relatively well advanced, but manipulating the tumor microenvironment through inhibiting other checkpoints is also demonstrating potential for enhancing other immunotherapeutics. Preliminary studies suggest blocking TIM3 can enhance cancer vaccine efficacy, at least in a prophylactic setting.[19]

Additional improvements can be achieved by blocking multiple checkpoints, as was observed in the study by Curran et al.,[18] when anti-CTLA-4 was added to the anti-PD-1, anti-PD-L1 plus Flt3L vaccine treatment regimen. Similarly, when both PD-1 and CTLA-4 inhibitory pathways were blocked, a better outcome was observed using IL-15 to treat intravenously injected CT26 metastatic colon cancer in mice.[20] Combining blockade of TIM3 and other checkpoints can also have enhanced anti-tumor

effects.[21] Indeed, there is evidence to suggest that further benefit can be achieved using more complex combinations. For example, optimal effects against melanoma in mice were demonstrated when anti-PD-L1 was combined with adoptive transfer of tumor-specific CD8+ T cells, DC-peptide vaccine, IL-2 and irradiation.[22]

Some recent clinical trials suggest that not all components of combination therapies are necessary. A Phase III clinical trial using check-point blockade (Ipilimumab) and/or a gp100 vaccine resulted in increased median overall survival in patients receiving the combined therapy, but this was not greater than those receiving Ipilimumab alone.[1]

Immune inhibition can also be mediated by adenosine generated within the tumor microenvironment by the action of CD73. Blockade of this immunoregulatory pathway can lead to increased activity of adoptively transferred T cells against CD73-expressing tumors.[23,24] In addition to tumor cells, CD73 can be expressed on endothelium, and inhibition of CD73 can increase T cell adhesion to endothelial cells and localization to tumors.[25]

Targeting Regulatory Cells

Many different cells with immunosuppressive potential can infiltrate the tumor environment, including M2 macrophages, Treg cells, and MDSCs. Suppressive mechanisms employed by these cells involve secretion of cytokines (e.g., IL-10 and TGFβ), secretion of enzymes (e.g., arginase, NOS and IDO), and expression of inhibitory receptors (e.g., CTLA-4 and PD-L1). Depleting or modifying these regulatory cells and targeting each of the mechanisms they use within the tumor microenvironment can reverse immunosuppression (**Fig. 1**). In combination with other immunotherapies, it can lead to enhanced tumor regression.

Blocking differentiation or recruitment. MDSC differentiation can be blocked using cyclooxygenase (COX) inhibitors, which prevent the production of prostaglandin.[26] In combination with tumor lysate-pulsed DCs, a COX inhibitor could decrease the immunosuppressive function of myeloid cells and improve the survival of tumor bearing mice.[27] All-trans retinoic acids (ATRA) have also been shown to reduce the presence of immature MDSC by converting them to non-immunosuppressive mature myeloid cells, thereby prolonging the anti-tumor effect of cancer vaccines.[28] Complete rejection of tumors was also achieved when CpG therapy was combined with an antibody blocking CCL1, neutralizing de novo conversion of Treg.[29]

In addition to abrogating the function of regulatory cells by blocking their differentiation, accumulation of regulatory cells in the tumor microenvironment can be reduced by targeting chemokine pathways. CCL2 for instance, is an important chemo-attractant for myeloid suppressor cells and its neutralization could augment the antitumor activity of vaccine[30] or adoptive CTL transfer.[31] Treg recruitment, through CCL17 and CCL22, could also be inhibited using a small molecule antagonist of CCR4, which led to improved responses to vaccine.[32]

Blocking immunosuppressive enzymes. The suppressive activity of myeloid cells has been associated with the catabolism of the amino acids arginine and tryptophan. Arginase (ARG) can deplete arginine, and indoleamine 2,3-dioxygenase (IDO) can
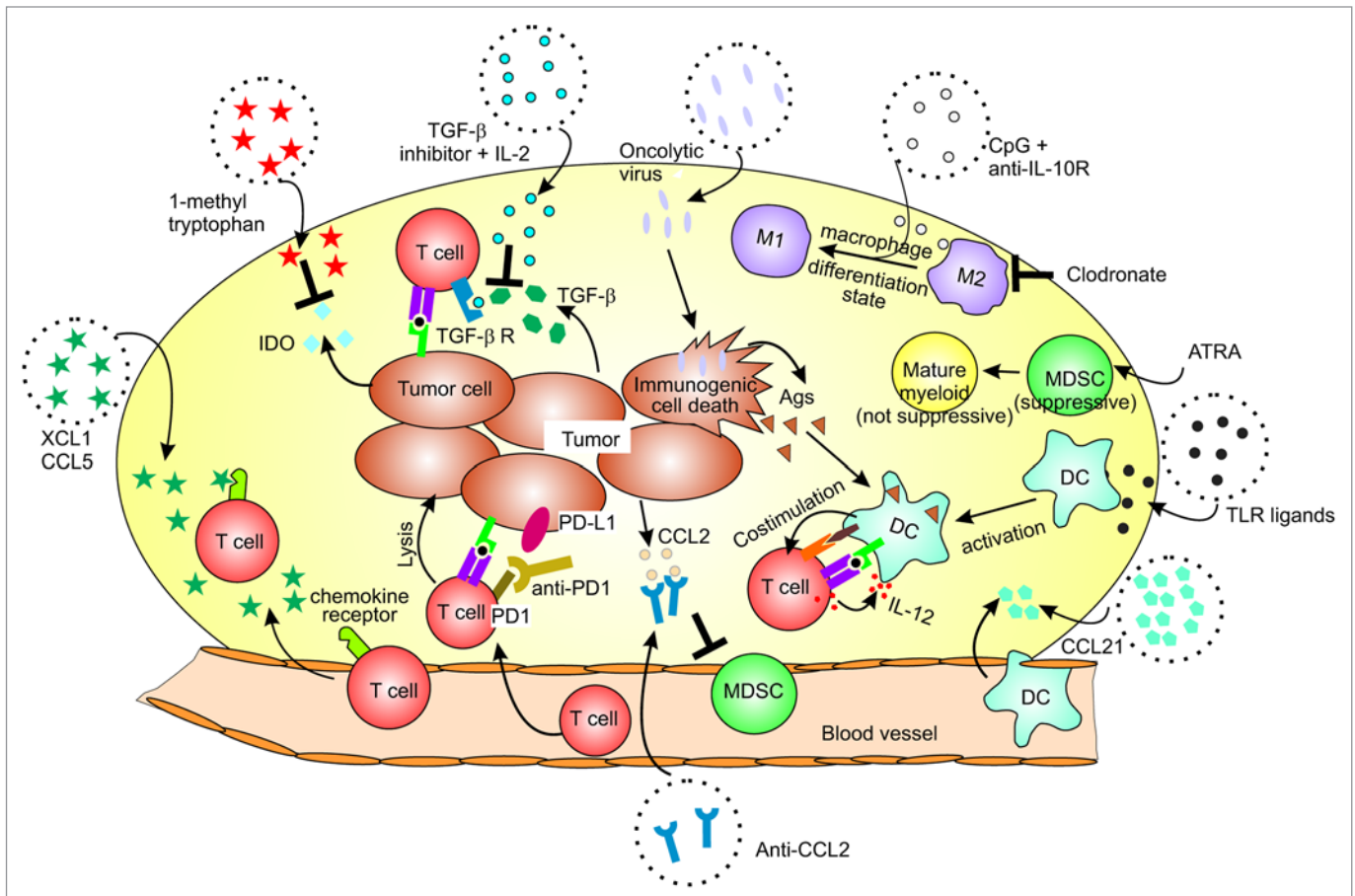
Figure 1. Immune modulators of the tumor microenvironment that enhance cancer immunotherapy. Different therapies are depicted as described in the text. ATRA, all-trans retinoic acid; IDO, Indoleamine 2, 3-dioxygenase; M1 or M2, M1 or M2 macrophage; MDSC, Myeloid-derived suppressor cell; PD-1, programmed cell death protein 1; TLR, Toll-like receptor

degrade tryptophan present in the tumor micro-environment.[33,34] Recently, ARG-expressing M2 have been targeted using *N*-hydroxy-L-Arg (NOHA) and survival of sarcoma tumor bearing mice have been increased when combined with αOX40 therapy.[35] Both ARG and NOS are blocked simultaneously by nitroaspirin or sildenafil (Viagra®). Combined with a tumor vaccine or adoptive T cell therapy, these molecules could reduce function of MDSC, increase number of tumor infiltrating specific CTL[36] and improve the survival of tumor-bearing mice.[37]

Several immunotherapies including the use of vaccines,[38–40] and cytokines[41,42] have been improved when used in combination with the IDO inhibitor, 1-methyl-tryptophan. Knockdown of IDO by small-interfering RNA has also demonstrated the benefit of IDO inhibition combined with immunotherapy. Efficacy has been demonstrated when it was directly loaded in DCs used as a vaccine[43] or used in combination with a DNA vaccine encoding the tumor-associated antigen HER-2.[44]

Regulatory cell depletion. Multiple regulatory mechanisms within the tumor microenvironment can be targeted simultaneously by depletion of subsets of regulatory leukocytes, which has been demonstrated to enhance immunotherapy in mouse models. Clodronate encapsulated in liposomes is a reagent

for the depletion of macrophages in vivo. This reagent can deplete M2 macrophages and increase the efficacy of therapies including anti-angiogenic therapy using anti-VEGF or α-CD137 and CpG combination immunotherapy.[45,46]

Monoclonal antibodies specific for Gr-1 could deplete MDSC, and combined with adoptive cell therapy,[47] or OVA protein based vaccine[48] or anti-VEGF antibody therapy[49] resulted in an enhancement of immunotherapy and regression of established tumors. Welford et al. showed that depleting Tie2-expressing proangiogenic macrophages from mammary tumors, through a suicide gene based strategy, improve the effect of a vascular disrupting agent.[50]

While M2 macrophages and MDSC can be found in large numbers in tumors and their immunomodulatory activity is often exerted locally within the tumor microenvironment, it is less clear where the immunoregulatory activity of Treg is performed since it can occur in lymphoid tissue and/or the tumor itself. Nevertheless, there are several approaches where depletion of Treg in tumors has enhanced immunotherapies. Several investigators have shown that depletion of Treg using anti-CD25 antibody can enhance the efficacy of a variety of immunotherapies.[51–53] However, the potential benefit of Treg depletion through

anti-CD25 antibody can be lost by the concurrent elimination of activated effector lymphocytes. In the DEREG mouse model, Foxp3^{+} Tregs express a diphtheria toxin receptor and so can be selectively eliminated with diphtheria toxin. The use of this model has already shown that elimination of Treg in tumors leads to tumor infiltration with CD8^{+} T cells and enhances survival of mice when combined with various types of vaccination.[54,55]

Re-programming immunosuppressive cells. An alternate, and more translationally feasible, approach to use instead of depleting regulatory cells is to re-program them to circumvent immunosuppression. Macrophages possess a certain degree of plasticity with regard to phenotype, and it is possible to manipulate tumor-associated immunosuppressive M2 macrophages to become immunosupportive M1-like. A range of strategies including CpG oligodeoxynucleotides combined with anti-IL-10 receptor and adenoviral delivery of CCL16 chemokine[56] or with anti-CD40 antibody and chemotherapy[57] or with anti-CD40 and anti-disialoganglioside[58] are able synergize in the induction of anti-tumor effects and to be associated with repolarization of tumor-associated macrophages. Immunotherapy using agonist anti-CD40 antibody combined with chemotherapy has shown remarkable effects in both mice and patients with pancreatic carcinoma by redirecting infiltrating macrophages to anti-tumor potential.[59]

Treg have been recently observed to possess an unexpected degree of phenotypic plasticity and could lose their suppressor phenotype and become reprogrammed into T helper-like cells. Combining IDO inhibitor and an anti-tumor vaccine caused upregulation of IL-6 in plasmacytoid DCs and in situ conversion of a majority of Tregs to a Th17 phenotype, with marked enhancement of CD8^{+} T cell activation and antitumor efficacy.[60,61] Furthermore, gemcitabine, in combination with a recombinant adenovirus expressing the tumor-associated antigen Her-2 and an anti-GITR antibody, was demonstrated to revert in vivo Treg immunosuppressive activity, achieving therapeutic effectiveness against pre-existing tumors.[62]

Modifying the Chemokine Profile of the Tumor Microenvironment

The cellular composition of tumors is influenced by the chemokine profile of the microenvironment. Individual types of leukocytes are attracted in response to specific chemokines. Manipulation of the chemokine makeup can be used to swing the balance of cell types, and their associated molecules, from immunosuppressive to immunopotentiating with anti-tumor activity. This section of the review highlights the variety of chemokines which are being exploited to manipulate and alter the microenvironment, and which are then used in conjunction with one or more other immunotherapies to improve localization of effector lymphocytes to tumors (**Fig. 1**).

CXCL10 and XCL1, which attract CD8^{+} T cells, NK cells and monocytes, are chemokines used in a number of studies. The intratumoral injection of adenovirus- or plasmid-encoded XCL1 has been combined with a variety of immunotherapies including adoptive transfer of effector T cells,[63,64] delivery of cytokines such

as IL-12[65,66] or IL-18,[67] and DC vaccine.[68] Together they caused considerable tumor regression or eradicated all tumors, which could include non-injected distant tumors, with a role for CD4^{+} and CD8^{+} T cells together with NK cells identified.

Other chemokines used to attract T cells into tumors include CCL5. An oncolytic vaccinia virus encoding CCL5 was given in combination with tumor lysate-pulsed dendritic cells to achieve greater tumor inhibition.[69] CCL2 has also been used to recruit T cells to tumor. Two herpes simplex virus vaccines were used in one study, one encoding the chemokine CCL2 and the other encoding IL-12. Intratumoral injection of these together resulted in significant tumor infiltration by CD8^{+} T cells and enhanced inhibition of neuroblastoma.[70]

Recruitment of DCs and monocytes to tumors can also enhance immunotherapy. CCL21 and CCL16 can attract DCs and macrophages in addition to T cells. CCL21 injected intratumorally in combination with injection of CpG-oligonucleotide[71] led to tumor inhibition associated with infiltration of CD4^{+} T cells and DCs. CCL16 delivered by adenoviral vector to the tumor has been used in combination with CpG and αIL-10 monoclonal antibody resulting in 60% of tumors rejecting both primary 4T1 tumors and distant metastases.[56] Macrophages shifted from an M2 to M1 phenotype intratumorally and DCs upregulated costimulatory molecules, secreting cytokines to stimulate T cell proliferation and activation.

Thus, chemokines can be a potent way of changing the cellular composition of tumors, although efforts to date have largely focused on attracting T cells. Future approaches may employ chemokines better suited to recruiting other leukocytes with anti-tumor potential. Indeed, harnessing response capabilities normally reserved for pathogens through Toll-like receptors (TLRs) can be a potent way of attracting a variety of leukocytes and triggering changes to the microenvironment.

Danger Signals (TLR)

TLR agonists can trigger broad inflammatory responses that elicit rapid innate immunity and promote the activation of the adaptive immune reaction.[72] Two of the most commonly used TLR agonists are Polyinosinic:Polycytidylic Acid [Poly(I:C)] and Cytosine-phosphorothioate-guanine (CpG) (agonists for TLR3 and TLR9 respectively). Intratumoral injection of CpG alone[73] or in combination with PolyI:C[74] enhanced the anti-tumor efficacy of adoptive transfer of tumor specific T cells. Stimulation of endogenous tumor immunity can also benefit from TLR agonist delivery. This was demonstrated using anti-CD137 together with local tumor injection of CpG that led to increased expression of genes associated with antigen presentation together with an increased frequency of tumor-infiltrating T cells, resulting in total regression of the majority of established tumors.[46] Enhanced antigen presentation was also thought to play a role in optimal antitumor effects observed when using intratumoral injection of CpG and poly(I:C) in combination with intratumoral delivery of CD40 ligand.[75]

Oncolytic viruses are highly immunogenic pathogens able to stimulate TLR, and because they infect or replicate predominantly

Table 1. Examples of strategies for manipulating the tumor microenvironment to enable immunotherapy

Strategy	Microenvironment modifier	Additional immunotherapy	Effect within tumor microenvironment	Effect on tumor size and mouse survival	Ref.
Check point inhibitors	PD-1 and CTLA-4 blockade	Anti-PDL1 plus vaccination with irradiated B16 tumor expressing Flt3L	Increased infiltration of T cells into tumor, IFNγ production, and ratio of effector T cells to MDSCs	65% rejection of s.c. B16 tumors.	18
	Anti-CD73	ACT of tumor-specific CTL	Enhanced accumulation of effector T cells in tumor, due to restored T cell adhesion and homing.	Delayed tumor growth and enhanced survival of mice bearing s.c. B16-SIY tumors.	25
Depletion of regulatory cells or inhibition of their suppressive effects	AT38 (blocks peroxynitrite produced by MDSCs)	ACT of tumor-specific CTL	Reduction in intratumoral nitrotyrosines and N-CCL2 expression, enhanced expression of CCL2, induced T cell infiltration.	Rejection of 60% of s.c. EG7-OVA and > 70% of s.c. MCA-203 tumors.	31
	Treg blockade with anti-CCL1	CpG-ODN	Decreased Treg numbers, increased tumoricidal T cells.	Complete tumor rejection in mice bearing s.c. TUBO tumors.	29
	IDO inhibitor of Treg suppressive function	IL-12 + GM-CSF microspheres	Transient reduction in Tregs, and increase in ratio of CD8+ to T suppressor cells.	Tumor rejection in 45% of mice bearing metastatic intramammary 4T1 tumors.	41
Modifying chemokine profile	Oncolytic vaccinia virus expressing CCL5	Tumor lysate-pulsed DCs	Enhanced homing of CD4+ and CD8+ T cells and NK cells, increased IL-12.	Delayed tumor growth of s.c MC38 tumors and enhanced survival of mice.	69
	Adenovirus expressing CCL16	CpG plus anti-IL-10R antibody	Accumulation of macrophages and DC intratumorally, reversing their immuno-suppression, enhanced TNF and IL-12 production.	Eradication of most tumors in mice bearing s.c tumors of TSA (90%), 4T1 (60%) or MC38 (74%).	56
Inflammatory mediators and Toll-like receptor agonists	Oncolytic vaccinia virus	Anti-CD137 agonist antibody	Increased infiltration of CD8+, NK cells and neutrophils.	Tumor eradication in > 35% of mice bearing s.c. AT3 tumors.	77
	HSV-TK retrovirus adhering to T cells	ACT of tumor-specific CTL + gancyclovir + lympho-depletion	Tumor heparanase expression ensured specific delivery of retroviral particles. Maximum number of T cells in tumor occurred at 72–96h.	90% survival of s.c. B16-OVA bearing mice when low numbers of T cells transferred.	81
Manipulating cytokines	IL-12 transgene in T cells	ACT of tumor-specific CTL + lymphodepletion	Reversed suppression of MDSCs and other immuno-suppressive myeloid cells in tumor.	20–40% survival of mice bearing s.c. B16 tumors.	96,97
	TGFβ inhibitor in liposomal gel (nLG)	IL-2 in nLG	Increased infiltration of NK cells and activated CD8+ T cells	40% survival of mice bearing s.c. B16F10 tumors	90

A variety of agents can be used to modify the tumor microenvironment as listed. Together with additional immunotherapies, effective anti-tumor responses can be mediated.

in tumor cells, much of their activity is localized to tumor. They can induce inflammatory mediators attracting myeloid cells and lymphocytes.[76] In addition, they can lyse a proportion of tumor cells, thereby releasing immunogenic antigens. In one study, oncolytic vaccinia virus was injected into subcutaneous tumors and combined with intraperitoneal delivery of anti-CD137. This combination therapy resulted in increased tumor infiltration by NK cells, neutrophils, and CD8^{+} effector T cells and enhanced inhibition of tumor growth and survival of mice.[77]

There are many other strains of oncolytic virus under development that may induce novel and desirable changes to the microenvironments of tumors in various locations. For example, oncolytic myxoma virus when injected intratumorally and combined with adoptively transferred tumor-specific T cells was demonstrated to lead to enhanced survival of mice with syngeneic B16-SIY melanoma brain tumors.[78]

In addition, studies have shown that loading antigen-specific T cells with viruses such as the vaccinia virus or vesicular stomatitis virus, have led to more efficient delivery of virus to tumor, resulting in increased T cell localization and activation, associated with effective tumor regression, and significantly increased survival.[79,80] The ability of retroviral particles to adhere to the surface of T cells was utilized enabling viruses encoding IL-12 or Herpes simplex thymidine kinase (HSVtk) to "hitchhike" on antigen-specific T cells, which were delivered by adoptive transfer. Between 5–14% of the injected dose localized to the tumor, curing 60–90% of mice carrying B16-OVA tumors.[81] Further elegant strategies like these may lead to more specific and effective delivery of both TLR agonists and other immune modulators to tumors.

Thus oncolytic viruses can trigger immunogenic cell death and inflammation that can lead to an enhanced immune response against cancer. Immunogenic cell death can also be mediated by triggering death receptors on tumor cells. Targeting one such receptor, using anti-DR5, on a variety of mouse tumors can synergize with immunotherapies that augment antigen presentation (CD40) and T cell costimulation (CD137/4–1BB) to eradicate established tumors.[82] Recent investigations also demonstrate that targeting DR5 can have profound effects on the tumor microenvironment by disrupting tumor vasculature, although this specific feature has not been investigated in combination with additional immunotherapy.[83]

Manipulating Cytokines in the Tumor Microenvironment

The cytokine content of the microenvironment can influence the balance of immunosuppressive and immunosupportive factors within tumors. Many types of immunotherapy can benefit from co-administration of cytokines, but delivery is often systemic making it difficult to distinguish between contributions from microenvironment modification and systemic immune modification. However, some studies have directed cytokines specifically to tumors and demonstrated changes

to the microenvironment and increased efficacy of additional immunotherapeutic agents. For example, T cell-mediated production of IL-12 within tumors has been demonstrated to reprogram immunosuppressive leukocytes to enable tumor destruction by adoptively transferred T cells.[84,85] Localized delivery of IFNα to tumors has also been demonstrated to enhance immunotherapies using a DC vaccine or agonist anti-CD137 antibody.[86,87]

TGFβ secretion within tumors can suppress the antitumor activity of leukocytes in the tumor microenvironment. Thus various inhibitors have been used in cancer therapy to block TGFβ and lift immunosuppression, while simultaneously delivering other immunotherapies to eradicate the tumor.[88] For example, TGFβ was inhibited by intratumoral injection of two inhibitory peptides which were combined with simultaneous i.t. injections of poly(I:C) and α-CD40 antibody.[89] The TGFβ inhibited was mainly that produced by Tregs rather than tumor cells, leading to 70% rejection of tumors in mice. Another innovative method of TGFβ inhibition involved nanoscale liposomal polymeric gels releasing a TGFβ inhibitor that, when combined with IL-2, significantly delayed B16 tumor growth in mice.[90] There were significantly increased numbers of intratumoral NK cells (required for maximal tumor regression) and intratumoral infiltration of activated CD8^{+} T cells, in parallel with demonstration of localized therapy and drug to the tumor.

Concluding Remarks

Thus, there are a variety of strategies that can be used to modify the tumor microenvironment to render it less immunosuppressive and enable additional immunotherapies (**Table 1**). The majority of the above studies were preclinical in mice, which allowed interpretation of mechanistic contributions to therapy, but these types of combination therapies are gaining momentum in the clinic.[91,92]

Checkpoint blockade has been used in combination with other immunomodulators, and benefit to patients demonstrated.[93] However, most clinical studies using combinations of immunotherapeutic agents have been early stage trials and mechanistic insight into the relative roles of immunomodulation and microenvironment modulation has not been possible. Nevertheless, there is much excitement in the use of combination therapy with remarkable response rates reported in a recent clinical study by combining anti-CTLA-4 and anti-PD1.[94] More complex combinations are possible,[95] although careful evaluation of potential toxicities prior to commencing clinical trial is crucial to the safety of these studies.

In summary, immunotherapy of cancer can induce anti-tumor responses, although these responses are not often complete. The immunoregulatory nature of the tumor microenvironment can inhibit fully effective immune responses against cancer, and modulation of the microenvironment can enhance the efficacy of immunotherapy to achieve eradication of tumors.

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