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The Suppressive Tumor Microenvironment: A Challenge in Cancer Immunotherapy

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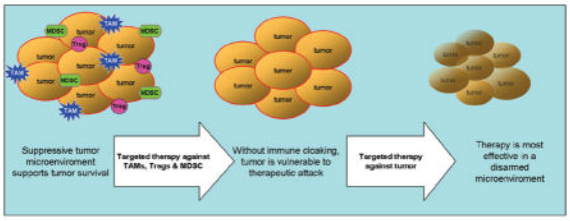
Abstract

In this review, we introduce the changing public perception of vaccines and immunotherapy in cancer treatments. We discuss the roles that different immunosuppressive cells play in the tumor microenvironment. Tumor associated macrophages (TAMs) and M1 and M2 macrophage phenotypes are discussed in depth. Additionally, the role that myeloid derived suppressor cells (MDSC) and T regulatory cells (Tregs) play in the tumor microenvironment is addressed. Highlighted are examples of therapies used against each suppressive cell type, which vary from the hypothetical to the ineffective; the inefficient to the successful. A variety of treatments have been tried to combat this fundamental problem, indeed the cause that allows cancerous mutated cells to survive, multiply and overtake the body. Efficient methods to disable each particular suppressive type of cell have been introduced; this review summarizes the discussion with a table to guide future development. We see gene therapy as the most innovative and flexible method to lead the charge to specifically modifying the tumor microenvironment.

Graphical abstract



Keywords

therapeutic vaccine; immunotherapy; tumor associated macrophages (TAMs); myeloid derived suppressor cells (MDSC); T regulatory cells (Tregs)

1. INTRODUCTION

In the past few years, the public's perception of vaccines has radically changed. The retraction of Andrew Wakefield's landmark 1998 publication in the Lancet that had

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correlated the development of autism with vaccinations and primarily the preservative thimerosal had less impact than a scientist could hope. Rather, the emergence of preventative vaccines such as Gardasil and Cervarix, which vaccinate against strains of the human papillomavirus (HPV) attributed to cause 70% of cervical cancers, and sipuleucel-T (APC8015, Provenge), a therapeutic vaccine that utilizes personalized medicine to treat prostate cancer, have created renewed energy and interest in vaccines and immunotherapy, particularly for cancer treatment.

Gardasil and Cervarix are *preventative* "cancer vaccines". They are approved to prevent HPV infection (and subsequent transformation of cervical epithelial cells (or any others) into cancer) and primarily accomplish this through humoral immunity. Both formulations deliver protein antigens from HPV capsid proteins to alert the immune system to the appearance of the virus. However, after the cells are transformed, the infected cells no longer produce the capsid proteins ("L" or late proteins), but rather early proteins ("E"), and the vaccine is not effective in patients that have already been infected.

In regards to adjuvants, Gardasil uses aluminum hydroxide. Until recently, alum was the only approved FDA adjuvant, utilized in nearly every marketed vaccine. AS04 is the second adjuvant to be approved in the US, and only appears in one vaccine (Cervarix). It builds upon the success of aluminum hydroxide, quite literally, with monophosphorylated lipid A conjugated to aluminum hydroxide.

Sipuleucel-T (APC8015), with the brand name Provenge, is the first ever approved *therapeutic* cancer vaccine: a treatment for prostate cancer. The drug is a cellular based therapy, removing patients' peripheral blood mononuclear cells (PBMCs), stimulating them with an antigen-cytokine fusion protein and reinjecting the cells (now activated against the tumor antigen, in this case, prostatic acid phosphatase).[1,2] Granulocyte macrophage-colony stimulating factor (GM-CSF) acts as the adjuvant in this drug and is the cytokine included in the fusion protein that the PBMCs are treated with. However, because the drug is a fusion protein, GM-CSF is not classified as an adjuvant in the same way that aluminum hydroxide and AS04 are classified.

However, even with the recent successes, a good cancer vaccine has a great deal to overcome. It is not adequate to simply contain a strong adjuvant, specific antigen and particle form to make it attractive to the immune system. In some cases, an effective Th1 response can be measured (via IFN-γ levels), however, the tumor remains.[3] Tumor cells use many mechanisms to avoid detection, including downregulation of MHC class I,[4] Fas and DcR3,[5] PD-L1[6] and secretion of a myriad of cytokines.

Stromal cells have a strong impact on the tumor microenvironment and how the immune system interacts with the tumor.[7] Several different types of hematopoietic nonvascular stromal cells can be present and protect the tumor from discovery and elimination, such as tumor-associated macrophages (TAMs), myeloid derived suppressor cells (MDSC), and T regulatory cells (Tregs) by secretion of cytokines. Removal or suppression of these inhibitory cells can enhance the tumor recognition and regression by the immune system.

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This review will present different types of hematopoietic nonvascular stromal cells and their effect on the tumor microenvironment, as well as strategies to overcome these effects.

2. TUMOR ASSOCIATED MACROPHAGES (TAMS)

2.a. Overview

Tumor-associated macrophages (TAMs) support tumor growth by secreting cytokines and growth factors that nurture the growing tumor cells.[8] Macrophages are also often described by their phenotype instead of primary location, however, TAMs and M2 macrophages share many of the same characteristics, if not identical.[8] The M2 macrophages can be thought of as “healer” macrophages “protecting against autoimmunity” that often secrete IL-10 and transforming growth factor-β (TGF-β) and act in an immunosuppressive manner. Contrastingly, the M1 macrophage is remembered as the “killer phagocyte”, most often playing an immunostimulatory or inflammatory role, producing inducible nitric oxide synthase, IL-12 and TNF.[9,10] In the studies presented here, in the text of the citations the original authors may refer to the cells as TAMs and the cells show aspects of M2 phenotype, however we have reproduced the terminology with which the original authors described their work.

IL-10 and TGF-β are well established M2 macrophage cytokines, even so far as to define the phenotype, and they will not be discussed in depth here. Indeed, even if TGF-β were deterred, the M2 phenotype remains. Recent work has shown the emergence of another cytokine that is crucial for TAM suppression of the tumor. The loss of TGF-β signaling has led to an upregulation in a chemokine named CCL2 (or MCP-1, monocyte chemotactic protein-1).[11]

Chemokine (C-C motif) ligand 2 (CCL2), also known as monocyte chemotactic protein-1 MCP-1), has a strong influence in the recruitment of macrophages and lymphocytes into tumor interstitium. This chemokine is produced by macrophages and some tumors and has been shown to recruit macrophages and lymphocytes into the tumor microenvironment, and it increases tumor growth and metastasis.[11–16]

Increased levels of cellular stress from, for example, smoking or cancer have been shown to sensitize some cells to produce moreCCL2 when stimulated with LPS. Compared to baseline, CCL2 serum levels increased 28.5-fold in non-small cell lung cancer patients, 15-fold in healthy smokers and 13-fold in the group of nonsmokers, when peripheral blood mononuclear cells (PBMCs) were stimulated with LPS.[13] This sensitivity could explain the difference in the role of macrophages in a tumor microenvironment compared to a less severe inflammatory environment.

CCL2 human gastric carcinoma model transfectants induced tumor growth, tumorigenicity, lymph node metastasis, ascites, TAMs in and around tumors, and significantly higher microvessel density.[14] In a xenograft prostate cancer model, CCL2 was shown to contribute to the regulation of TAM infiltration and enhanced angiogenesis within the tumor.[15] CCL2 concentration has been shown to increase with tumor stage (severity of tumor) and correlate with TAM accumulation in human colorectal cancer.[12,16]

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The concept of positive feedback is an important one to consider with chemokine induction in the tumor microenvironment. In biopsies of human head and neck squamous cell carcinoma, TAMs secreted CCL2 as well as IL-6.[17] Both cytokines have been shown to upregulate antiapoptotic proteins and inhibit the caspase cascade.[18] If both the tumor and the TAMs are secreting CCL2, there is an amplification loop[19] to recruit more and more macrophages to the tumor and surrounding area, increasing the negative effects from the secreted cytokines and chemokines by the macrophages. In fact, IL-6 and CCL2 themselves have been shown to promote mutual induction, protection from apoptosis and polarization toward the M2 type macrophage.[18] Knockout of HIF-1α in TAMs also induces M2 polarization.[20]

2.b. Solving the Tumor Associated Macrophage (TAM) Problem

Inhibition of CCL2 has been of great interest lately as many negative effects have been elucidated regarding TAMs and CCL2. Gene therapy delivered to a human malignant melanoma (B16-F1) has shown that delivering a dominant negative CCL2 mutant gene (7ND) overexpressing 7ND protein reduced TAMs, tumor angiogenesis, and tumor growth. Also, levels of TNFα, interleukin-1α (IL-1α) and vascular endothelial growth factor (VEGF) decreased in the tumor, attributed to fewer TAMs infiltrating the tumor.[21] In another study, inhibition of CCL2 in human melanoma xenografts reduced tumor growth and macrophage recruitment resulting in necrotic tumor masses.[22]

In one case, codelivery of CCL2 DNA and a cytotoxic treatment to the tumor showed suppression of colon cancer growth inmice.[23] Recombinant adenovirus (rAd) for herpes simplex virus thymidine kinase (HSV-tk) was delivered plus ganciclovir, and in another virus capsid, an rAd expressing CCL2 was delivered. The authors hypothesize the regression was due to infiltrating monocytes and macrophages as well as Th1 cytokines and CTL activity. In this case, the cytotoxic activity of the HSV-tk would cause local inflammation and indeed apoptotic bodies. With the codelivery of CCL2, M1 macrophages would be recruited to the tumor, prepared to engulf and present the antigens to enhance the immune response. However, this recruitment into the tumor was not seen when CD80 DNA was delivered in a rAd compared to CCL2 DNA.

Ability to switch the phenotype from M2 to M1 would solve the TAM problem and allow the infiltrated macrophages to become powerhouses of destruction. One study observed such a change by delivering an adenovirus containing CCL16 (AdCCL16) and CpG intratumorally and the anti-IL10R antibody via an intraperitoneal injection.[24] A few hours after treatment, tumor macrophages switched from M2 toM1 type as measured by the secretion of TNF, IL-12, and nitric oxide. Even dendritic cells in the tumor had upregulated costimulatory molecules and secreted IL-12 and TNF. The presence of inhibitory M2 type macrophages was reemphasized as the delivery of AdCCL16 alone was not able to cause tumor regression. The inclusion of CpG (a TLR9 agonist) and anti-IL10R antibodies were crucial for the phenotype flip. Thus, with the inhibition of the IL-10 signaling (suppression of the M2 phenotype[25]), and activation of macrophage and dendritic cells via TLR9 (sign of the M1 phenotype[26]), the cells were able to transfer from one phenotype to the other. However, one weakness of this study is the delivery aspect, with three separate injections

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needed (by two different delivery routes) to create the desired effect, therefore more work should be done to further refine the delivery of this treatment.

An indirect targeting method could be implemented, taking advantage of the freely circulating immune system and alerting the system as a whole to the surface molecules on TAMs. When dosed with a DNA vaccine for legumain, an asparaginyl endopeptidase overexpressed by TAMs, a TAM specific T cell response was observed, a decrease in TAMs and an increased number of mice surviving a lethal tumor challenge.[27]

There is also potential to solve the TAM problem using a direct targeted approached for M2 cells. The conversion to an M2 phenotype caused by CCL2 or IL-6 shows a significant increase in the mannose receptor (CD206).[18] Mannose can be conjugated or incorporated to treatments to target therapies to M2 polarized macrophages. Directly targeting M2 cells would be the ideal treatment modality to allow flexibility in payload of drug. However, direct delivery to TAMs targeting via mannose has not yet been published.

3. MYELOID DERIVED SUPPRESSOR CELLS (MDSC)

3.a. Background

Myeloid derived suppressor cells (MDSC) are often CD11b and Gr-1 positive, but are broadly defined and have many functions.[28] Not only are these two markers found on neutrophils, but also immature dendritic cells, monocytes and early myeloid progenitors. MDSC are immunosuppressive, and also endorse neovascularization.[29] In humans, MDSC have been reported to stain $CD34^+CD33^+CD13^{+}$.[30] When directly exposed to a murine ovarian cancer cell line, MDSC express high levels of CD80 (but not CD86).[31] As with all immune cells, new subpopulations are constantly being defined and redefined; MDSC are no different, with mononuclear or MO-MDSC, and low-density polymorphonuclear cells or PMN-MDSC. MO-MDSC was shown to function through STAT1, IFN-γ or nitric oxide (NO), whereas PMN-MDSC required IFN-γ, and ROS but not STAT1 or NO.[28,32] Other reviews have named these two groups differently, assigning the abbreviations M-MDSC and G-MDSC, for monocytic and granulocytic MDSC, respectively.[33]

The appearance of MDSC has been documented in many different cancers including mouse mammary carcinoma (MMC),[34] 4T1,[34,35] and murine colorectal (MC38/CEA2).[36] It has been shown in breast cancer that $CD11b^+Gr\text{-}1^+$ are recruited into tumors with type II TGF-β receptor gene (*Tgfbr2*) deletion.[35] Recruitment of MDSC to tumors has also been shown by several different chemokines including CXCL12, SDF-1α, CCL2, CXCL5 and KIT ligand.[37] Secretion of TGF-β1 by MDSC in the tumor microenvironment has been shown to promote metastasis in breast cancer.[35]

Immature myeloid cells inhibit $CD8^+$ T cells in direct cell–cell contact, show increased amounts of reactive oxygen species (ROS) caused by accumulation of H_2O_2. Also, MDSC have increased ROS production in the presence of stimulated Ag-specific T cells (stimulated with their specific Ag and producing IFN-γ).[38] MDSC are inversely correlated with NK function in the liver and the spleen and have been shown to inhibit NK cell production of IFN-γ by MDSC membrane-bound TGF-β1.[39]

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In a tumor that produced IL-1β, accumulation of $CD11b^{+}Gr\text{-}1^{+}$ cells correlated with poor prognosis.[40] Not only does IL-1β attract MDSC to tumors, but GM-CSF and VEGF as well. $CD34^{+}$ cells showed accumulation in Lewis lung carcinoma tissues selectively (rather than the surrounding tissues), entirely attributed to the secretion of VEGF.[41] High expression of GM-CSF in the tumor causes increased numbers of MDSC and immunosuppression.[42,43]

Not surprisingly, MDSC do not act alone. MDSC and macrophages communicate in the tumor via cell–cell contact, with macrophage production of IL-12 decreasing dependent on MDSC production of IL-10.[44] The overexpression of CD80 on MDSC allowed for the suppression of antigen specific immune responses in concert with T regulatory cells (see below).[31]

3.b. Solving the Myeloid Derived Suppressor Cell (MDSC) Problem

The simplest fix is to remove the draw that recruits the MDSC into the tumor interstitium in the first place. Resection of large tumors producing IL-1β restored immune activity, while treatment with IL-1R agonist reduced tumor growth.[40]

Another less elegant solution is to remove the suppressive cells from the body, and convert them *in vitro* to a tumor fighting immune cell type. Isolated $CD11b^{+}Gr\text{-}1^{+}CD31^{+}$ suppressor cells can be converted *in vitro* to myeloid dendritic cells with exposure to IL-4 and GM-CSF.[43] This could facilitate removal of the suppressive MDSC, *ex vivo* priming of the newly formed dendritic cells with tumor antigen, and redosing of the activated DCs to the patient. However, an effective method to extract the MDSC from the tumor interstitium does not yet exist, and thus the need for new and innovative treatments to combat MDSCs.

Formalin-inactivated herpes simplex virus (HSV) delivered to a murine system of colorectal cancer inactivated MDSC through B cells. It was shown that expansion of MDSC required B cell production of angiogenesis factors such as VEGF-A, and neuropilin-1 (NRP-1).[45] Delivery of *all-trans*-retinoic acid (ATRA) to patients with metastatic renal cell carcinoma reduced the number of immature myeloid suppressor cells, if the patients retained a high plasma concentration of ATRA >150 ng/mL.[46]

Combination therapy is a popular approach to combat MDSC's effect on tumor growth. Delivery of IL-7 and IL-15 after radiofrequency thermal ablation reduced tumor growth, metastasis and numbers of MDSC in murine breast tumors.[34] As another example, the delivery of 5-fluorouracil and leucovorin together with a DC vaccine reduced the numbers of both MDSC and T regulatory cells (another suppressive immune cell to be discussed in detail later) in a murine colorectal cancer model.[36] Decrease of MDSC in the microenvironment and tumor control of colorectal tumors (inoculated with intrahepatic injection) was achieved by delivery of an adenovirus coding IL-12 in conjuction with oxaliplatin. The IL-12 expression cassette was developed to be mifepristone inducible, thereby allowing control of IL-12 dosing. With this treatment, an increase in the CD8+/Treg ratio was also observable.[47]

While this review has focused on the effect of regulating these suppressive cells in the tumor microenvironment, it is useful to note that eradication of the suppressive cells from

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anywhere in the body can elicit a therapeutic response. Gemcitabine was able to decrease splenic MDSC in large tumor bearing mice, while increasing the antitumor activity of $CD8^+$ T cells and NK cells.[44,48] This was further augmented by an intratumoral injection of adenovirus containing IFN-β.[48]

4. $CD4^+CD25^+FOXP3^+$ REGULATORY T CELLS

4.a. Overview

T regulatory cells (Tregs) are positive for surface markers CD4, CD25 (IL-2 receptor) and an intracellular marker, FoxP3. Additionally, these cells have high surface expression of cytotoxic T-lymphocyte-associated antigen 4 (CTLA-4) and glucocorticoid-induced TNFR-related protein (GITR).[49] These cells have been found to accumulate in metastatic melanoma tumors and prevent tumor regression, even when CTL are present.[50] Although their function has not been completely elucidated, the presence of Treg cells have been linked to poor tumor regression and can be induced by immunostimulatory treatments.[51,52]

Presence of Tregs in tumor tissue is cancer specific. Early stage non-small cell lung cancer and late-stage ovarian cancer patients were first observed to have Treg infiltration.[53] Tregs are rarely seen in enucleated choroidal melanoma,[54] however they are found in human prostate cancer and are more common in the peripheral and transition zones than in the core of the tumor.[55] In gliomas, infiltration of Tregs was correlated with heme oxygenase-1 (HO-1) mRNA[56] and progression of the disease. Increased populations of Tregs have been found in hepatocellular carcinoma, and even in nontumorous liver that contains primary hepatic tumors.[57]

Tregs have been shown to migrate toward IL-6 and IL-8-producing tumors by induction of CXCR1 by IL-6.[58] Knowing that TAMs produce IL-6 may support more than one suppressive stromal cell employed by the tumor at a time. Similarly, TAMs and tumor cells producing CCL22 recruited $CCR4^+$ Tregs in ovarian cancer and glioblastomas.[59,60] NK depletion in mice bearing Lewis lung carcinoma (LLC) has shown increase in expression of CCL22 and correlated increased levels of Tregs.[61] In both human and mouse models of pancreatic cancer, tumors produce CCL5 that recruits $CD4^+FoxP3^+$ Tregs to the tumor.[62]

Tregs contain high levels of cytotoxic T lymphocyte (CTL)-associated antigen 4 (CTLA-4) which can bind with CD80/86 on APCs, which can cause production of indoleamine 2,3-dioxygenase (IDO), which interferes with the activation of T cells. Some types of Treg cells have been defined as secreting IL-10 and TGF-β but not IL-4 or IL-2.[63] IL-10 production can inhibit proliferation of $CD4^+$ cells.[64] The exact mechanism of suppression is unknown, and the myriad of phenotypes makes specification difficult, however, it is well established that presence of Tregs in tumor interstitium correlates with poor prognosis.

4.b. Solving the Regulatory T Cell (Treg) Problem

Preclinically, delivery of anti-CD25 antibodies has shown success in increasing the life span of tumor bearing animals in a variety of tumor types (glioma, pancreatic).[56,65,66] In one study of glioblastomas, survival was found to be tumor burden dependent, indicating that even if Tregs are eliminated there is a limit to the strength of an unstimulated immune

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response.[66] In this same study, the use of PC61 (an anti-CD25 antibody) completely inhibited clonal expansion of tumor antigen-specific T cells. As CD25 is also on activated T cells, the delivery of anti-CD25 antibodies inhibited the tumor-specific CTLs, which should be a reminder in drug delivery to be cautious when dealing with dual targets (on the suppressive and killer cells).

Daclizumab is a humanized monocolonal antibody against CD25. In a small clinical trial, it has been used in metastatic breast cancer patients to clear $CD25^{+}Fox3^{+}$ cells from circulation, to allow for a more preferable environment to dose a peptide vaccine and elicit a CTL response.[67] However, the outcome of this drug's effect on overall survival or progression free survival in humans is yet to be determined conclusively. Daclizumab is still being tested in clinical trials for many types of cancers, including: two types of glioblastoma (www.clinicaltrials.gov; NCT00626483, NCT00626015), ovarian cancer (NCT01132014), breast cancer (NCT00573495), hematologic cancers (NCT00006350, NCT00019305, NCT00001941, NCT00001249, NCT00002681), and melanoma (NCT00847106, NCT01307618).

A simpler way to target CD25, the IL-2 receptor, is with IL-2. Denileukin diftitox is a fusion protein combining IL-2 and diphtheria toxin. Tregs were decreased in peripheral blood samples after multiple doses of denileukin diftitox, enhancing the efficacy of a dendritic cell vaccine.[68] In treating melanoma, after dosing with denileukin diftitox, numbers of Tregs, $CD4^{+}$ and $CD8^{+}$ T cells decreased, but T cell repopulation included $CD8^{+}$T cells specific for melanoma antigens. After at least one cycle of denileukin diftitox, five out of sixteen patients experienced melanoma regression, some with metastatic lesions (NCT002996889).[69] When denileukin diftitox was compared against cyclophosphamide and anti-CD25, cyclophosphamide provided the greatest decrease in Tregs, but also reduced the population of $CD8^{+}$ T cells. Denileukin diftitox reduced more than half of the Tregs, with anti-CD25 having a lesser but longer lasting effect.[70]

Other experimental treatments have varied approaches and outcomes. With the positive effect CD25 agonists and antibodies have demonstrated, it is not surprising anti-CTLA4 antibodies were also tested. Treatment consisting of chronic administration of anti-CTLA4 antibodies was unable to deplete Tregs or impair function in a B16 melanoma model, and actually allowed the population of Tregs to expand in percentages and absolute numbers, but was effective at causing tumor regression when coupled with a GM-CSF transducer tumor cell vaccine.[71] However, anti-CTLA4 has shown efficacy as a single agent in humans, as ipilimumab, a human monoclonal antibody against CTLA4 was approved for unresectable or metastatic melanoma by the FDA on March 25, 2011. A simple vaccine of DOTAP and peptide antigen has been shown to decrease the number of Treg cells and cause regression of existing tumors.[51] In one case, the addition of low dose CpG to an immune stimulatory complex (ISCOM) vaccine decreased the population of Tregs. Delivery of a CCR5 inhibitor (TAK-779) in pancreatic adenocarcinoma limits Treg migration to the tumor, as well as producing tumors smaller than those in control subjects.[62]

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5. CONCLUSION

With the growing promise of gene delivery, and the plasticity of immune cells and lineage, it is important to consider the target genes that could be modified to reverse the suppressive immune environment. Table 1 notes several genes discussed in this review that play important roles in the immune regulation of the tumor microenvironment. As can be seen, most of the proposed strategies involve using siRNA to downregulate the target gene. The only upregulation desirable is the expression of a dominant negative mutant of CCL2. Thus, delivery of specific siRNA or plasmid DNA to the immune cells in the tumor interstitium is highly desirable. It presents a new challenge to the gene therapy community.

While a great deal of work has been done in creating treatments to overcome the tumor microenvironment, a great deal still remains. While all cell types reviewed in this review deal with hematopoietic stromal cells, a recent publication in *Science* by Kraman and colleagues explores mesenchymal stromal cells and the inhibitory nature with the expression of fibroblast activated protein-α (FAP-α).[72] The study showed that the removal of FAP-α expressing cells permits the efficacy of a therapeutic vaccine in a solid tumor model. While the model of expression is transgenic and artificial, the importance of FAP-α lends insight to yet another method utilized by tumors to prevent immune recognition. Whatever the method of immune suppression the tumor employs, it seems that any therapeutic cancer vaccine that hopes to be efficacious must simultaneously be able to switch the immune system on, and switch the tumor microenvironment off.

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Table 1

Target Genes in the Suppressive Tumor Microenvironment

gene/protein	target cell(s)	strategy
CCL2	TAMs	siRNA to ↓ expression
IL-6	TAMs	siRNA to ↓ expression
dominant negative CCL2 mutant gene (7ND)	TAMs	gene transfer to ↑ expression
CCL16	TAMs	gene transfer to ↑ expression
IL-10	TAMs, Tregs	siRNA to ↓ expression
heme oxygenase-1 (HO-1)	Tregs	siRNA to ↓ expression
transforming growth factor-β (TGF-β)	TAMs, MDSC, Tregs	siRNA to ↓ expression
STAT1	MDSC	siRNA to ↓ expression
indoleamine 2,3-dioxygenase (IDO)	APCs	siRNA to ↓ expression
IL-6, IL-8, CCL22	tumor (with goal to prevent TAM recruitment)	siRNA to ↓ expression
CXCL12, SDF-1α, CCL2, CXCL5, KIT ligand, IL-1β, GM-CSF, VEGF	tumor (with goal to prevent MDSC recruitment)	siRNA to ↓ expression
CCL5	tumor (with goal to prevent Treg recruitment)	siRNA to ↓ expression