

Tumor-Specific Cytotoxic T Cells Are Crucial for Efficacy of Immunomodulatory Antibodies in Patients with Lung Cancer

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Abstract

There is growing evidence that activation of the immune system may be an effective treatment for patients with either small cell lung cancer or non-small cell lung cancer (NSCLC). Immunomodulatory antibodies directed against cytotoxic T cell-associated antigen 4 (CTLA-4/CD152) and programmed cell death ligand 1 (PDL1/CD274) showed clinical efficacy in patients with lung cancer. The key immune cells responsible for antitumor activity are the CTLs. The presence of these tumor-directed CTLs, both in number and functionality, is a prerequisite for the immune system to attack cancer cells. Immunomodulatory agents attempt to increase the efficacy of CTL activity. Thus, the limited number of patients who benefit from immunomodulatory antibodies may be caused by either an inadequate number or the impairment of CTL activity by the hostile environment created by the tumor. In this review, we discuss tumor-induced impairment of CTLs and experimental treatments that can stimulate T-cell responses and optimize specific CTL function. We discuss 2 types of immune cells with known suppressive capacity on CTLs that are of pivotal importance in patients with lung cancer: regulatory T cells and myeloid-derived suppressor cells. *Cancer Res*; 73(8); 2381–8. ©2013 AACR.

Introduction

There is growing evidence that approaches that activate the immune system may be effective treatment options for patients with either small cell lung cancer (SCLC) or non-small cell lung cancer (NSCLC). Immunomodulatory antibodies against cytotoxic T cell-associated antigen 4 (CTLA-4/CD152) and programmed cell death ligand 1 (CD274) showed clinical efficacy in patients with SCLC and NSCLC (1–4). On the basis of these early trials, phase III trials in lung cancer are currently enrolling patients. Table 1 summarizes the published data on these agents in patients with lung cancer.

However, important lesions learned from the studies in patients with melanoma must be taken into account: Data on anti-CTLA-4 treatment in advanced melanoma show that a subset of patients (5.8%–22%) has a marked improvement in survival, whereas in the majority of patients, the survival benefit is only modest or even absent. Apart from the high costs, treatment with these immunomodulatory antibodies is associated with specific side effects such as colitis and hypophysitis. The toxicity profile in patients with lung cancer is similar to that in other malignancies. Therefore, it is highly

desirable to predict which patients might benefit from immunomodulatory treatments.

The key immune cells responsible for antitumor activity are the CTLs directed against tumor cells susceptible to cell lysis (5). The presence of antitumor CTLs, both in number and functionality, is a prerequisite for the immune system to attack cancer cells. Immunomodulatory agents attempt to increase the efficacy of CTL activity. Thus, the limited number of patients that benefit from immunomodulatory antibodies may be caused by either the limited number of CTLs or impairment in CTL activity caused by the hostile tumor environment.

Absent or low CTL responses must first be induced or enhanced by passive or active immunotherapy. In lung cancer and mesothelioma, different approaches have been used to enhance CTLs (6). For example, we have shown that immunotherapy using *ex vivo* stimulated dendritic cells can induce antitumor immune responses in patients with mesothelioma (7).

The function of CTLs is affected by the systemic and local immunosuppressive environment created by the tumor (Fig. 1). Lung cancer is known to highly suppress the host immune response (6). This may also be enhanced by exposure to cigarette smoke and its effect on the immune system (8). Also, each tumor has its own set of genomic and epigenomic changes, which will influence the host immune response to the tumor (9). At present, the clinical impact of the summation of these changes in lung cancer is unknown.

Optimizing tumor-specific CTL function before administering immunomodulatory antibodies seems to be an essential step to increase the efficacy of these agents in lung cancer treatment. We anticipate that the efficacy of these new immunotherapeutic strategies will benefit from, first, the induction of powerful CTL responses, and second, by creating an environment that does not exhaust the function of tumor-specific CTL activity.

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Table 1. Published efficacy data on immunomodulatory antibodies in lung cancer

Reference, N	Disease	Treatment arm	Study design	Efficacy	Study result
Reck et al. (3), 130	SCLC Stage IV First line	Anti-CTLA-4 Ipilimumab	Randomized phase II Chemotherapy + placebo	irPFS	n.s. HR = 0.64; <i>P</i> = 0.03
			Ipi 10 mg/kg concurrent Ipi 10 mg/kg phased		
Lynch et al. (2), 204	NSCLC Stage IV First line	Anti-CTLA-4 Ipilimumab	Randomized phase II Chemotherapy + placebo	irPFS	n.s. HR = 0.72; <i>P</i> = 0.05
			Ipi 10 mg/kg concurrent Ipi 10 mg/kg phased		
Topalian et al. (4), 76	NSCLC Stage IV ≥Third line	Anti-PD-1	Phase I monotherapy	RR	95% CI 6 (0.1–27) 32 (13–57) 18 (8–34) 18 (11–29)
			1 mg/kg		
			3 mg/kg		
			10 mg/kg		
Topalian et al. (4), 18	Squamous Stage IV ≥Third line	Anti-PD-1	Phase I monotherapy	RR	95% CI 0 50 (12–88) 43 (10–82) 33 (13–59)
			1 mg/kg		
			3 mg/kg		
			10 mg/kg		
Topalian et al. (4), 56	Nonsquamous Stage IV ≥Third line	Anti-PD-1	Phase I monotherapy	RR	95% CI 0 23 (5–54) 13 (4–30) 12 (5–24)
			1 mg/kg		
			3 mg/kg		
			10 mg/kg		
Brahmer et al. (1), 49	NSCLC Stage IV ≥Third line	Anti-PD-L1	Phase I monotherapy	RR	95% CI 0 8 (0–36) 16 (5–36) 10 (3–22)
			1 mg/kg		
			3 mg/kg		
			10 mg/kg		
Brahmer et al. (1), 13	Squamous Stage IV ≥Third line	Anti-PD-L1	Phase I monotherapy	RR	95% CI n.d.p. 0 (0–60) 13 (0–53) 8 (0–36)
			1 mg/kg		
			3 mg/kg		
			10 mg/kg		
Brahmer et al. (1), 36	Nonsquamous Stage IV ≥Third line	Anti-PD-L1	Phase I monotherapy	RR	95% CI n.d.p. 11(0–48) 18 (4–43) 11 (3–26)
			1 mg/kg		
			3 mg/kg		
			10 mg/kg		
Abbreviations: CI, confidence interval; ipi, ipilimumab; irPFS, immune related progression-free survival; n.d.p., no data published; n.s., not significant; RR, response rate.					

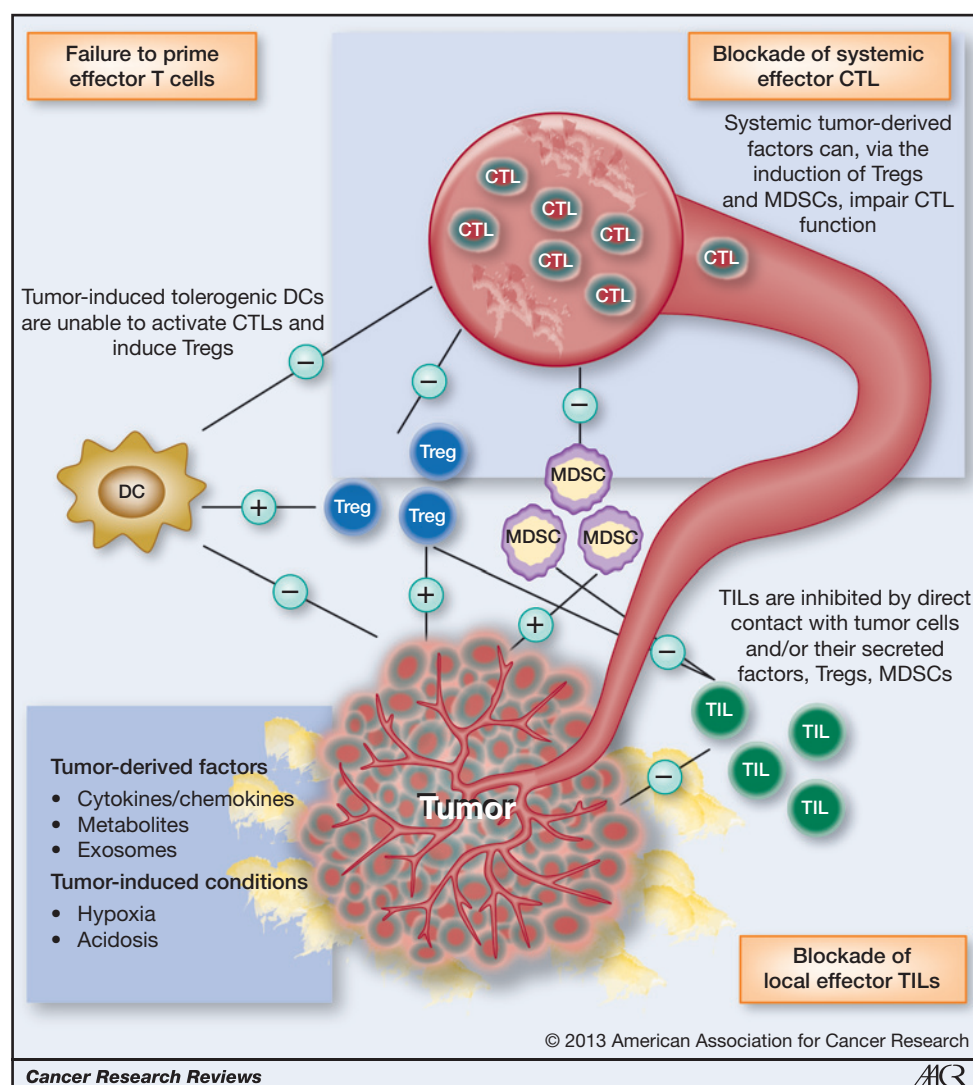
In this review, we discuss tumor-induced impairment of CTLs and experimental treatments that can stimulate T-cell responses and optimize specific CTL function. We discuss 2 types of immune cells with known suppressive capacity on CTL that are of pivotal importance in patients with lung cancer: regulatory T cells (Treg) and myeloid-derived suppressor cells (MDSC). We anticipate that strategies that enhance CTL activity in an immunostimulatory environment can strengthen the effectivity of immunomodulatory antibodies for more

patients. These conditions can reduce tumor burden in advanced lung cancer, whereas patients with limited-stage disease may even be cured.

Tumor-Induced Impairment of T Cells

The coevolution of tumor cells and their surrounding stroma generates an environment capable of suppressing effector T cells in number and activity, thereby contributing to cancer progression (10). This immunosuppression, thus orchestrated

Figure 1. Immune evasion of the tumor by the impairment of effector T cells. The expansion of effector T cells may be prevented by downregulation of DC function. T cells are forced in an anergic tolerogenic or exhausted stage by tumor-derived factors, immunosuppressive cells (e.g., Tregs, MDSC), and direct contact with tumor cells (e.g., death receptor ligand expression, galactins). TIL, tumor-infiltrating lymphocyte.



by the tumor or engendered by surrounding cells in response to the tumor, has recently been added as a new hallmark for cancer (11, 12). Tumors use at least 2 strategies to prevent destruction by effector T cells; first they avoid recognition by downregulating key molecules [e.g., MHC class I molecules, tumor-associated antigens (TAA)], making them poor stimulators and targets for tumor-specific T cells. Second, tumors and their environment can prevent T-cell activation, disable their function, or induce T-cell apoptosis. This changing of tumor characteristics in time demands a continuous adaptation of the immune system. Figure 1 shows the currently known mechanisms for a tumor to evade antitumor T-cell responses.

Failure to prime the immune system

Tumor antigen presentation by antigen-presenting cells (APC) in draining lymph nodes is the first and an essential step in the expansion and differentiation of antitumor CTLs. CTLs are released into the circulation and migrate into tumor tissue [then called tumor-infiltrating lymphocytes (TIL)] by complex interactions with adhesion receptors under the influ-

ence of chemotactic factors (Fig. 1). The most important APC to activate T cells is the dendritic cell (DC). Under normal conditions, DCs play an important role in the immunosurveillance of the host by initiating this primary antitumor effector T-cell response. Also, in the activation of the adaptive immune system against tumor cells, DCs play a crucial role, as they are able to engulf tumor antigens and activate naïve lymphocytes in an antigen-specific manner. However, tumor cells are potent sources of a wide variety of cytokines, chemokines, and metabolites that interfere with the natural development and function of circulating and local DCs. Furthermore, tumors create conditions like hypoxia that stimulate immunosuppressive cells like macrophages to acquire a proangiogenic M2 phenotype with high production of proangiogenic factors like VEGF and matrix metalloproteinase (MMP-9; ref. 13). Tumor-derived factors such as VEGF and interleukin (IL)-10 cause both numerical and functional defects of DCs. These DCs express substantially lower levels of MHC molecules, adhesion molecules, and costimulatory molecules and have impaired capabilities for antigen uptake, diminished cell motility, and

impaired ability to activate naïve T cells (14–20). Although tumor-infiltrating DCs are scarce in human tumor biopsies, their presence in tumors correlates with a better prognosis (21–23). A pilot study in patients with lung cancer showed that the percentages of immature DCs and the phenotype of CTLs in blood are related (24).

Blockade of effector phase

Direct interaction between tumor cells and TILs may result in the downregulation of the CD3 ζ chain and specific tyrosine kinases of the TIL. This contributes to an impaired T-cell receptor signaling that inhibits CTL lytic function and inactivates the effector phase of antitumor responses. It has been suggested that the expression of death receptor ligands (e.g., FasL, TRAIL, RANTES) by tumor cells or on their secreted exosomes may deliver death signals to activated T cells, even triggering T-cell death at distant sites from the tumor (25).

The plethora of tumor-secreted factors can also negatively affect T-cell survival, activation, and cytokine secretion. For example, glycan-binding proteins such as members of the galectin family expressed by tumor cells and cancer-associated stroma can bind to glycoconjugates on T cells, delivering signals intracellularly, thereby inhibiting T-cell effector functions by inducing T-cell apoptosis, sensitizing T-cells to FasL-induced cell death, blocking proximal T-cell receptor (TCR) signals, and suppressing T_H1 and proinflammatory cytokine secretion. Also, other pleiotropic factors, such as IL-10, TGF- β , and prostaglandin E2 (PGE2), can directly inhibit T-cell activation, proliferation, and differentiation.

Several immunologic checkpoints are built in to switch off T-cell activity. CTLA-4, lymphocyte activation gene-3 (LAG-3), T-cell immunoglobulin mucin-3 (TIM-3), and programmed death-1 (PD-1) are examples of these coinhibitory molecules to regulate T-cell accumulation and effector function. The engagement with their ligand usually leads to the attenuation of T-cell responses against cancer cells. Sustained signaling results in functional exhaustion of T cells, during which the ability to proliferate, secrete cytokines like IL-2, and mediate lysis of tumor cells is sequentially lost. Furthermore, tumor-derived factors and/or conditions can also induce and attract immunosuppressive cell types such as Tregs and MDSCs (26). This important mechanism by which effector T cells are attenuated by these cell types is discussed in more detail below.

Regulatory T cells

Tregs are a subpopulation of CD4⁺CD25⁺ T lymphocytes that are produced during maturation in the thymus (natural Tregs) or induced in the periphery (induced Tregs). They have a pivotal role in maintaining homeostasis and immune quiescence and are therefore an important "self-check" to prevent excessive reactions. In humans, these cells represent 2% to 5% of total circulating CD4⁺ T cells in peripheral blood. Defects in Tregs contribute to the induction of severe autoimmune diseases, including rheumatoid arthritis. They are further characterized by the expression on forkhead box P3, glucocorticoid-induced TNF-receptor-related protein (GITR), LAG-3, CTLA4, and downregulation of CD127 (IL-7R); however, all

these markers are not truly Treg specific. Induced Tregs arise from naïve T cells when triggered by suboptimal antigen stimulation by tolerogenic DCs and stimulation with TGF- β and IL-10. Induced Tregs can be subdivided into IL-10-secreting Tregs type I, TGF- β -producing Tregs (T_H3 cells), and IL-35-secreting Tregs (27). These cells are characterized by the secretion of immunosuppressive cytokines directly inhibiting effector T cells and converting DCs into suppressive APCs (28). This contagious spread of suppressive capacity, mainly mediated by IL-35, from Tregs to other T cells is called infectious tolerance (29).

In patients with cancer, Tregs confer growth and metastatic advantages by inhibiting antitumor immunity. They have this protumoral effect by promoting tolerance via direct suppressive functions on T cells or via the secretion of immunosuppressive cytokines such as IL-10 and TGF- β (30, 31). Tregs are increased in tumor tissue (32) and in peripheral blood (33) of patients with NSCLC compared with healthy volunteers. This increase in Tregs was found to promote tumor growth, correlated with lymph node metastasis (34, 35), and the number of Tregs is associated with poor prognosis (36). Many factors lead to increase of Tregs in NSCLC tumors; among them are thymic stromal lymphopoietin (37) and intratumoral COX-2 expression (38). Overall, Tregs are considered the most powerful inhibitors of antitumor immunity (39). As a result, there is substantial interest for overcoming this barrier to enhance the efficacy of effector T cells. Strategies include the following: (i) Treg depletion by chemical or radiation lymphoablation or using monoclonal antibodies or ligand-directed toxins directed against CD25 (daclizumab, basiliximab, denileukin diftitox, RFT5-SMPT-dgA, and LMB-2) or with metronomic cyclophosphamide; (ii) suppression of their function [ipilimumab, tremelimumab (anti-CTLA4), DTA-1 (anti-GITR), denosumab (anti-RankL), modulation of Toll-like receptor, OX40 stimulation, or inhibiting ATP hydrolysis using ectonucleotidase inhibitors]; (iii) inhibition of tumoral homing by blocking the selective recruitment and retention of Tregs at tumor sites (e.g., CCL22, CXCR4, CD103, and CCR2); and (iv) exploitation of T-cell plasticity by modulating IL-6, TGF- β , and PGE2 (e.g., the COX-2 inhibitor celecoxib) expression (40).

Myeloid-derived suppressor cells

MDSCs are a heterogeneous population of immature myeloid cells and myeloid progenitor cells. In healthy individuals, MDSCs generated in bone marrow quickly differentiate into monocytes, granulocytes, or DCs. However, not only in cancer (including NSCLC; ref. 41) but also in patients with trauma, sepsis, and in autoimmune diseases, a partial block in the differentiation results in expansion and release in the peripheral circulation of MDSCs. They are dramatically increased in blood, spleen, and at tumor sites in patients with advanced malignancies. MDSCs have been shown to inhibit the activation of T cells (42, 43) in a nonspecific or antigen-specific manner, alter the peptide presenting ability of MHC class I molecules on tumor cells (44), inhibit the DC differentiation (45), and expand Tregs (46, 47), signifying their contribution in constituting a tumor-suppressive environment. Furthermore, there is compelling evidence that MDSCs, by secreting MMP-9

and TGF- β 1, are also involved in angiogenesis, vasculogenesis, and metastatic spread (48).

They suppress these features by the production of reactive oxygen species (ROS), nitric oxide, peroxynitrite, and secretion of the cytokines IL-10 and TGF- β (49). Upregulated arginase-I activity by MDSCs depletes the essential amino acid L-arginine, contributing to the induction of T-cell tolerance by the down-regulation of the CD3 ζ chain expression of the TCR (50–53). However, the mechanisms that are used to suppress the immune responses are highly dependent on the context of the microenvironment (54).

An increased subpopulation of MDSCs in the peripheral blood of patients with NSCLC was detected that decreased in those patients who responded to chemotherapy and patients undergoing surgery (55). Because MDSCs play an important role in mediating immunosuppression on CTLs, they represent a significant hurdle to successful immunotherapy in NSCLC.

Therefore, targeting MDSCs *in vivo* with drugs like 5-fluorouracil, gemcitabine, or VEGF/c-kit blockers (e.g., sunitinib, imatinib, dasatinib) to elicit more potent anticancer effects is an exciting development (56–58). Treatment of mice with all-*trans*-retinoic acid, along with the help of NK T cells, converts the poorly immunogenic MDSCs into fully efficient APCs and in this way reinforces antitumor immune responses (59). Other MDSC-suppressing or differentiation-inducing agents recently reported are 5-aza-2'-deoxycytidine, curcumin, IL-10, anti-IL4R aptamer, and vitamin D3 (60–62). Agents that decrease arginase activity, ROS, and/or inducible nitric oxide synthase (iNOS) expression by MDSCs include Nor-NOHA, 1-NMMA, COX-2 inhibitors (celecoxib; ref. 63), phosphodiesterase-5 inhibitors (sildenafil, tadalafil; ref. 64), or ROS inhibitors (nitroaspirin; ref. 65). Because some of these agents are approved by the U.S. Food and Drug Administration for non-cancer-related indications, they promise to be a fruitful avenue of investigation in the coming years to overcome immunosuppression associated with advanced tumors (56, 57).

CTL activation

Cancer immunotherapy attempts to activate or enhance the antitumor effects of the immune system of the patient. Multiple approaches for immunotherapy have been developed to improve antitumor T-cell responses, and many are in various stages of (pre)clinical research. These approaches can be divided into 2 categories: passive and active immunotherapy.

Passive immunotherapy. Passive immunotherapy makes use of *in vitro* produced immunologic effectors that are capable of influencing effector T-cell responses. The most common forms of passive immunotherapy are the administration of recombinant cytokines or monoclonal antibodies. Cytokines such as IL-2, IL-7, IL-15, and IL-21 that bind to the common γ -receptor on T cells (IL-2RG) can expand T cells *in vivo* and increase the persistence. Monoclonal antibodies are also used as immunomodulators to inhibit immunosuppressive molecules/cells or activate immunostimulatory molecules. Examples are antibodies directed against CTLA-4, PD-1, PD-L1, TIM-3, or BTLA that can prevent blocking of T-cell

effector functions. Anti-CTLA-4, anti-PD-1, and anti-PD-L1 have been studied in clinical trials in patients with lung cancer (Table 1). On the basis of these data, these agents are now being tested in phase III studies both as first-line (anti-CTLA-4) and later-line treatments (anti-PD-1). The toxicity profile for these agents in patients with lung cancer is similar to that for other malignancies, but as experience with these agents is increasing, earlier interventions for immune-related toxicities (e.g., treatment of colitis with high doses of steroids) seems to reduce the amount of grade 3 and 4 toxicity.

Another way of passive immunotherapy is to reduce the suppressive environment by depleting VEGF, for instance, with bevacizumab, a monoclonal antibody against VEGF. An additional advantage could be that the DCs become better T-cell activators by this treatment.

Active immunotherapy Active immunotherapeutic approaches aim at inducing or boosting immune effector cells *in vivo* through the administration of immune mediators or cells capable of activating the immune system. One method uses adoptive transfer of T cells after expansion and/or activation *ex vivo* (66). This approach of adoptive T-cell transfer to eradicate malignancies is rather challenging (67), mainly owing to the relatively small amount or absence of TILs in tumor tissue and peripheral blood CTLs. However, adoptive transfer with *ex vivo* expanded autologous T cells engineered with TCR or chimeric antigen receptor could then be a promising alternative when tumor antigens are known (68). Although antigen-specific cell transfer precludes their limited presence by the inhibitory effect of the immunosuppressive environment, there are limitations to the antigen diversity and specificity of these cells.

The aim of immunotherapy is to specifically enhance the immune response directed to the tumor. Therefore, defined TAA epitopes have been used to vaccinate patients with NSCLC (68); however, this approach is limited by the relatively low number of identified specific peptides for lung cancer and by the requirement of MHC typing. By using the whole TAA protein for immunization, the need of peptide identification may be circumvented. These proteins can be taken up by the APCs and endogenously processed into epitopes for presentation to T cells, among others. However, as antigens are taken up from the tumor cells, the above mentioned antigen specificity limitation is precluded. Therefore, the application of DC in therapeutic cancer treatment holds promise, but as described above, the function of APCs *in vivo* is hampered (69). DCs can be stimulated *in vivo*, but the clinical efficacy of this stimulation was found to be limited. However, it is possible to stimulate DCs *ex vivo* and then inject them into the patient. Recently, in metastatic castration-resistant prostate cancer, *ex vivo* cultured DC-based immunotherapy prolonged survival (70), providing evidence for cell-based immunotherapeutic agents in solid tumors. The disadvantages of DC-based immunotherapy are that it is laborious and expensive. However, properly stimulated DCs will activate diverse cell types and can generate CTLs with multiple specificities when loaded with broad-spectrum antigens.

Future Perspectives

At present, immunotherapy is taking its first steps into lung cancer treatment. The immune system has an extremely complex way of acting on signals with activating and suppressing interactions between cells and consists of many regulatory mechanisms. Certainly, in patients with cancer, the tumor influences this complex interplay. Therefore, theoretical and preclinical data cannot be automatically transferred to patients with cancer.

In this review, we have described the most important mechanisms hampering an effective antitumor T-cell response. It is evident that approaches to increase T-cell expansion, homing, and effector functions while simultaneously targeting immunosuppression are needed. Preclinical and clinical studies on immunotherapy mostly aim at one specific cell type, thereby neglecting the complex interplay between the different cell types.

Both in lung cancer and in mesothelioma, we and others have shown that immunotherapeutic approaches increase the activation of the good-natured immune system of the patient determined through a number of different laboratory techniques. To determine the clinical impact of this laboratory measurement is rather difficult. For clinical studies, it is difficult to determine the efficacy of immunotherapy, as reviewed by Madan and Gulley (70).

The complex interplay between tumor cells, the different immune cells, and the patient has only been acknowledged in the past few years. The objective in this would also be to maximally influence the tumor microenvironment with minimal systemic effects.

In our view, failure to prime the effector T cells can be circumvented by DC vaccination or adaptive T-cell therapy. To prevent blockade of the effector T cells, these therapies can best be combined with immunomodulatory antibodies like CTLA-4 or approaches that influence the immunosuppressive cell types. The best combination of treatment will depend on tumor- and patient-specific circumstances in time.

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Consequently, immunomodulatory antibodies should be administered when tumor-specific CTLs are present. If the number of CTLs is insufficient, they should either be induced by immunotherapy and/or by decreasing the immunosuppressive environment. Preclinical studies by our group support the concept that modulating the immunosuppressive environment in combination with therapies that simultaneously stimulate effector cells has clinical potential (63, 71).

Conclusions

The role of modulating the immune system of the patient in the treatment of lung cancer is evolving. Studies to increase CTL activity in lung cancer showed benefit in a subset of patients. We anticipate that effective immunomodulatory treatment is associated with high numbers of CTLs as well as optimal functional activity. Both characteristics are influenced by the systemic as well as the local immunosuppressive environment that is created by tumors.

To maximize the clinical applicability of immunomodulatory antibodies, future strategies must initially be aimed at increasing CTLs both in number and functionality and minimizing their impairment by the immunosuppressive environment.

Disclosure of Potential Conflicts of Interest

J. Aerts has received honoraria for service on the speakers' bureau and is a consultant/advisory board member of Roche. No potential conflicts of interest were disclosed by the other author.

Authors' Contributions

Conception and design: J. Aerts
Development of methodology: J. Aerts
Acquisition of data (provided animals, acquired and managed patients, provided facilities, etc.): J. Aerts
Analysis and interpretation of data (e.g., statistical analysis, biostatistics, computational analysis): J. Aerts
Writing, review, and/or revision of the manuscript: J. Aerts, J. Hegmans
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