

Immunotherapy prospects in the treatment of lung cancer and mesothelioma

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Abstract: A very recent finding is the role of immune activation in cancer. The assumption that stimulating the patient's immune system to attack tumors is a valuable treatment option in malignant diseases has gained more acceptance. However the high immunosuppressive effects caused by the tumor limits this beneficial effect. There is a delicate balance between immunoactivation and immunosuppression in a patient. Especially in non small cell lung cancer (NSCLC), the role of immunosuppressive cells hampering immune activation is high. But also in small cell lung cancer (SCLC) and mesothelioma immunosuppressive activity is high. This is suggested to be related to the type of tumor, advanced stage of the disease, and the tumor load. In this review, we provide an overview of the progress and challenges in the immunotherapeutic approaches in lung cancer. We conclude with the concept that immunotherapy in thoracic malignancies must be tailored made to the balance of the immune system.

Keywords: Non small cell lung cancer (NSCLC); immunotherapy; immune checkpoint antibodies



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Introduction

Lung cancer

Around 85% of lung cancers are non-small cell lung cancer (NSCLC), which includes three principal histological subtypes: squamous cell carcinoma, adenocarcinoma and large cell carcinoma (1). The treatment of NSCLC is based on the patient's clinical signs and symptoms, tumor stage and subtype, medical and family history, and data from imaging and laboratory evaluation.

The majority of patients (70%) is diagnosed with advanced (stage IV) disease, and until recently, palliative chemotherapy with platinum doublet therapy was the optimal treatment for these patients (2-4). However, chemotherapy has a limited impact on long-term survival of NSCLC and the five-year survival rate is extremely poor (5).

Recently the concept of driving mutations in NSCLC has dramatically changed the field of lung cancer treatment. Identification of these genotypic anomalies including

activating mutations and fusion genes has set the stage for personalized medicine for distinct subsets of genetically defined NSCLC (6). It involves tailoring treatment according to the genetic profile and molecular makeup of each patient, depending on the availability of targeted drugs. To date, several prognostic and predictive mutations have been identified in NSCLC; including oncogenic activation of epidermal growth factor receptor (EGFR) (HER1/ErbB-1); translocation of EML4-ALK or CD74-ROS; point mutations in *BRAF*, *PIK3CA*, and *MEK1*; amplification of *MET* (7-10). Patients with mutations have benefited from the development of target-specific therapy; e.g., gefitinib or erlotinib are effective EGFR tyrosine kinase inhibitors; crizotinib is used for ALK activation and sunitinib can be used when PDGFR is amplified (11,12). Objective response rates of 55% to 90% percent are observed when patients were selected based upon molecular criteria (8).

One of the most disappointing findings is the fact that tumors develop resistance to these agents (13). The

development of this resistance can either be mutation dependent, for instance genetic alteration of the drug target, or mutation independent, for instance via transformation of histology.

Small cell lung cancer (SCLC) accounts for approximately 15% of the lung cancer cases (14,15). In general, SCLC is initially sensitive to chemotherapy and radiotherapy (16). However, responses are often short-term and recurrence rates are high (16,17). Unfortunately, approximately 70% of patients diagnosed with SCLC have extended disease at presentation (18). These patients are treated with platinum-doublet chemotherapy and have a median survival of 10-12 months (19,20). The development of targeted therapies for SCLC has proven to be challenging, mainly due to the complex and not fully uncovered biology of SCLC (20,21).

Malignant mesothelioma

Malignant mesothelioma is a highly aggressive cancer caused by neoplastic transformation of mesothelial cells that line the body's serous cavities and the internal organs. In the majority of mesothelioma patients the tumor is localized within the pleural cavity. With a median survival of 9-12 months after first signs of illness, the prognosis is poor. Chemotherapy has shown to moderately prolong survival in these patients, while combined modality approaches, such as extrapleural pneumonectomy followed by radiochemotherapy, is only of benefit in a highly selective patient population (22,23).

The limited treatment options and poor prognoses of lung cancer and mesothelioma emphasize the need for novel treatments. Therefore, immunotherapeutic approaches are being investigated in these thoracic malignancies and an overview of these efforts is included in this review.

Complex relationship between the immune system and cancer

In 2007, Koebel *et al.* demonstrated that tumors induced in mice by the chemical carcinogen methylcholanthrene can be controlled by the host immune system (24). Suppressing the activity of the immune system allowed dormant tumors to wrest themselves from immune control and start dividing, disseminating and ultimately causing death of the host. Assumptions that the immune system plays a role in eradicating cancerous lesions or maintains them in a state of dormancy have a history back to 1909 by Paul

Ehrlich and by Lewis Thomas and MacFarland Burnet in 1957. The immunosurveillance concept is now accepted by the scientific community and "avoiding immune destruction" is included as the latest hallmark of cancer (25). Outgrowth of a tumor is divided in three phases often referred to as the three E's (Elimination, Equilibrium, Escape) of immunoediting. In the first phase, tumor cells are recognized by the immune system and eliminated or controlled in their growth. In the equilibrium phase the immune system iteratively selects and/or promotes the generation of tumor cell variants with increasing capacities to survive immune attack. In the escape phase the immunologically sculpted tumor expands in an excessive manner leading to physical symptoms of cancer by the host (26). It is important to note that at this third stage, the tumor and the immune system have been causing distorted immune system's cytotoxic activity, either by immunosuppressive activity or shedding of tumor antigens.

Setting the stage for immunotherapy

Developments of therapeutic antibodies, cancer vaccines, and cell-based immunotherapeutic approaches reveal both the promise and relative infancy of these agents to extend the life of patients with cancer. In 2010, sipuleucel-T (Provenge; Dendreon Corporation) received the first FDA approval of a cancer vaccine for the treatment of metastatic castration-resistant prostate cancer (27). It employs an adjuvant component to enhance the function of antigen presenting cells and immune effectors such as T cells. This was followed with the FDA approval in 2011 of the drug ipilimumab (Yelvoy, Bristol-Meyers Squibb) for the treatment of metastatic melanoma through potentiating T cell activity (28). Both agents, whose activity is discussed in more detail below, demonstrate improved survival in randomized phase 3 trials and reignited enthusiasm for the field of active immunotherapy. With the many clinical programs currently underway, new approvals for therapeutic cancer vaccines by FDA and other ruling authorities as EMA are expected in the coming years. Immunotherapy is now considered as the third wave in cancer therapy after conventional treatments and targeted agents.

Types of immunotherapeutic approaches

Immunotherapy attempts to stimulate or restore the body's natural ability of the immune system to fight cancer. There are various strategies to activate the immune system and

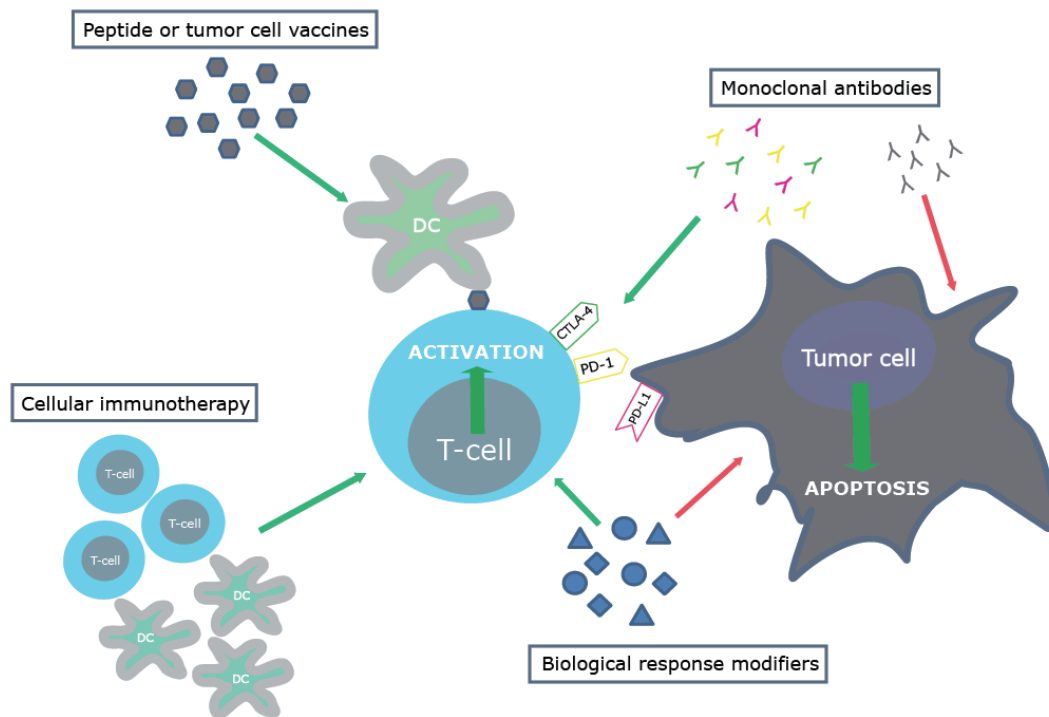


Figure 1 Immunotherapeutic approaches. Biological response modifiers are compounds which can specifically enhance the immune response, either by directly stimulating the immune system and/or by the direct induction of tumor cell apoptosis. These compounds can activate the anti-tumor immune response via the direct stimulation of pro-inflammatory immune cells or via the inhibition of detrimental suppressive immune cells like Tregs or myeloid-derived suppressor cells. Monoclonal antibodies bind specifically to one epitope and their application as potential immunotherapeutic agents has received a lot of attention recently. The development of antibodies which bind to co-inhibitory molecules activated during T-cell activation has led to the possibility to prevent T-cell inhibitory mechanisms and therefore enhance the anti-tumor immune response (29). Monoclonal antibodies targeting tumor growth related antigens (like EGFR) may diminish tumor growth by blockade of this receptor. On the other hand the IgG side of the antibody may cause immune-activation and tumor cell destruction in an immune related way. Monoclonal antibodies can also scavenge immunomodulatory proteins like VEGF. Peptide or tumor cell vaccines are designed to deliver tumor antigens to APCs, which can subsequently induce a tumor specific immune response by the adaptive immune system. These vaccines can consist of tumor specific antigens or can be composed of manipulated tumor cells. Cellular immunotherapy includes the adoptive transfer of autologous or allogeneic activated immune cells. The goal of this approach is to induce a tumor-specific immune response via the infusion of, e.g., tumor-antigen loaded dendritic cells or specifically activated T-cells.

these are classified here into the following categories: biological response modifiers, monoclonal antibodies, peptide or tumor cell vaccines, and cellular immunotherapy (Figure 1 and Table 1). There is no consensus regarding which of the four categories is the optimal approach for thoracic malignancies, this will probably be highly dependent on the tumor characteristics of each individual patient.

Within this research field, there is much attention for activating effector and memory T-lymphocytes because the release of their cytotoxic granules containing perforin and granzymes upon stimulation can lead to death of tumor

cells by apoptosis. Indeed, the infiltration of lung cancer with effector T-cells (CD3+CD8+) and memory T cells (CD45RO+) is associated with longer disease-free survival and/or a better overall survival in NSCLC (108-113). However, many other leukocyte types infiltrate the tumor environment: natural killer (T) cells, neutrophils, B- and T-lymphocyte subsets, myeloid derived suppressor cells, macrophages and dendritic cells. Based on their functions, these cells can be divided into cells with a potentially positive impact on the antitumor response and cells with a detrimental effect. The net effect of the interactions between these various cell types and their secreted products within

Table 1 Immunotherapeutic approaches that have been tested or are currently under investigation for lung cancer and mesothelioma			
Approach types	Examples of clinical studies in lung cancer and mesothelioma	Mode of action	References
Biological response modifiers			
Triggering inflammation	PF-3512676 (CpG 7909)	Toll-like receptor 9 agonist	(30-33)
	CpG-ODN 2006	Downregulation of Tregs	(34)
Cytokine therapy	Bacillus Calmette-Guerin (BCG), Mycobacterium vaccae (SRL172)	Nonspecific immune stimulants now often tested as adjuvants	(35)
	IL-2 + tumor necrosis factor alpha (TNF- α) or interferon alpha (IFN- α)	Induces T-cell proliferation	(36,37)
	Interferon gamma (IFN- γ)	Induces tumor cell apoptosis	(38,39)
Colony-stimulating factors	Mda-7 (IL-24)	Mda-7/IL-24 induces tumor cell apoptosis and inhibits tumor angiogenesis	(40)
	Granulocyte colony-stimulating factor (G-CSF)	Treatment of chemotherapy-induced neutropenia	(41,42)
Multi-modal effectors	Multi-target VEGFR: thalidomide and analogues such as lenalidomide and pomalidomide	Besides anti-metastatic, anti-angiogenic also immunomodulatory drugs (43,44)	(45,46)
	Cyclophosphamide	Targets regulatory T-cells (47,48)	(47)
	Cyclosporine	Targets regulatory T-cells (49)	(50)
	Denileukin Diftitox	Targets regulatory T-cells (51) and cancer cells which express the IL-2 receptor	(52)
	Talactoferrin	Recombinant human lactoferrin, promotes innate and adaptive immunity against tumor cells in the gut-associated lymphoid tissue (53,54)	(55-57)
	Trabectedin (Yondelis)	Targets tumor-associated macrophages (58) and tumor cells	(59,60)
	All-trans-retinoic acid (ATRA)	Targets myeloid-derived suppressor cells (MDSCs)	(61)
Monoclonal antibodies			
Directed against tumor cells	Cetuximab, Panitumumab, Matuzumab, Necitumunab	Chimeric or fully humanized antibodies which target the epidermal growth factor (EGF) receptor on tumor cells	(62-64)
	Trastuzumab (Herceptin)	Anti-HER2, targets tumor cells which overexpress the human epidermal growth factor 2 (HER2) protein	(65-67)
	CAT-5001 (SS1P)	Anti-mesothelin immunotoxin, targets mesothelin expressed in malignant mesothelioma and lung adenocarcinoma	(68)
Directed against tumor products	Amatuximab (MORab-009)	Chimeric anti-mesothelin monoclonal antibody	(69)
	Bevacizumab	Slows the growth of tumor blood vessels by targeting the VEGF protein. Blockade of VEGF is also immunomodulatory	(70,71)
Immune checkpoint inhibitors	Anti-CTLA-4 (Ipilimumab/tremelimumab)	Prevents T cell inhibitory mechanisms and allows T cells to continue cancer cell destruction	(72,73)
	Anti-PD-L1 [(BMS-936559/MP-DL-3280A)/PD-1 (BMS-936558 (nivolumab)/MK3475 (lambrolizumab)]		(74,75)

Table 1 (continued)

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Approach types	Examples of clinical studies in lung cancer and mesothelioma	Mode of action	References
Peptide or tumor cell vaccines			
Vaccines	GVAX	GM-CSF gene-transfected tumor cell vaccine	(76,77)
	Belagenpumatucel-L (Lucanix)	Allogeneic tumor cell vaccine made with four irradiated NSCLC cell lines modified with TGF- β 2 antisense plasmid	(78,79)
	MAGE-A3 vaccine	Vaccine composed of MAGE-A3 protein and adjuvant AS15	(80)
	(L)-BLP-25 anti-MUC-1 (Stimuvax)	Vaccine which targets MUC-1 expressed on tumor cells	(81,82)
	TG4010	Vaccinia vector coding MUC1 and IL-2	(83,84)
	CimaVax EGF	Vaccine composed of human recombinant Epidermal Growth Factor (EGF) conjugated to a carrier protein	(85)
	WT1 peptide vaccine	Vaccine composed of four WT1 (Wilms' tumor suppressor gene) analogue peptides	(86)
	CRS-207	Live-attenuated <i>Listeria monocytogenes</i> vector encoding human mesothelin (87)	(88)
	Bec2/BCG	Induces anti-GD3 antibodies (overexpressed on 60% of SCLC patients) (89)	(90,91)
	GV1001	Vaccine which targets the telomerase peptide GV1001	(92)
Racotumomab (Vaxira)	Anti-idiotypic antibody which mimicks the NGcGM3 ganglioside that is expressed on multiple human cancers (93)	(94), ClinicalTrials.gov: NCT01460472	
	Tergenpumatucel-L	Vaccine composed of irradiated and gene-transfected lung cancer cell lines	ClinicalTrials.gov: NCT01774578
Cellular immunotherapy			
Dendritic cells (DCs)	<i>Ex vivo</i> generated DC-vaccines	Dendritic cells loaded with tumor antigens	(95-98)
T-cells	<i>Ex vivo</i> generated lymphokine-activated killer cells (LAK)	Autologous lymphokine-activated killer cells (99)	(100,101)
	Cytokine-induced killer cells (CIK)	Autologous cytokine-activated T-cells and NK cells (103)	(102)
	Activated T-cells	Adoptive transfer of activated T-lymphocytes	(104)
	Gamma delta T cells	Adoptive transfer of zoledronate expanded gamma delta T-cells	(105)
Natural Killer (NK) cells	NK cells	Adoptive transfer of allogeneic Natural Killer (NK) cells (106)	(107)

the environment of an established tumor participates in determining anti-tumor immunity, angiogenesis, metastasis, overall cancer cell survival and proliferation (114).

The generation of a tumor specific cytotoxic T-cell response is dependent on the recognition of tumor antigens

by the T-cell receptor (TCR). The TCR can only recognize tumor antigen peptides presented by the MHC molecule on the membrane of an antigen-presenting cell (APC) like the dendritic cell. In addition to specific antigen recognition, co-stimulation is required for activation of the T-cell (115).

This second signal is antigen nonspecific and is provided through the interaction between co-stimulatory molecules on the membrane of the APC and the T-cell. Both the TCR engagement and the co-stimulatory signal are necessary to stimulate optimal T-cell differentiation and proliferation. Antigen recognition by the TCR in the absence of the second co-stimulatory signal results in T cell anergy or apoptosis (116).

Therefore, in addition to the activation of the anti-tumor T-cell response, there is increasing interest to modify this immunological balance, e.g., by targeting immune suppressive cell types or factors.

Different approaches are currently studied to overcome the earlier described immunosuppressive environment and to enhance the cytotoxic T-cell response. We developed dendritic-cell based therapy with the intention to potentiate the anti-tumor immune response and ultimately improve outcome in mesothelioma patients. It was demonstrated that this approach was safe and effective in mesothelioma patients (95,117).

Immunotherapy in lung cancer and mesothelioma: the hurdles. Data of our own research

Our institute has experience in immunological research in mesothelioma and lung cancer. We study the role of the immune system both in a preclinical and clinical model with focus on therapeutic interventions in patients.

We observed both in an animal model and in patients that the immune activity is prohibited via the upregulation of the number of immunosuppressive cells, which will be discussed below. We did find that the immune activity in patients with lung cancer or mesothelioma is often profoundly inhibited by a large array of immunosuppressive mechanisms (118-120). Regulatory T-cells (Tregs) and myeloid-derived suppressor cells (MDSCs) were found to be increased in the peripheral blood of lung cancer and mesothelioma patients (30). In addition, within the tumor anti-tumor T-cell responses were additionally inhibited by tumor-associated macrophages (121). In order to improve T-cell activity we have studied in our animal model whether modulating the suppressive function of Tregs (122), MDSCs (123) and macrophages (121,124) could improve immunotherapeutic approaches. In general we found that targeting these immunosuppressive cells has beneficial effects on the capacity of the immune system to be activated.

The plethora of immunosuppressive mechanisms is tumor-stage dependent. In our murine mesothelioma model, the immune activity correlated with tumor load (117). Also in patients with lung cancer this seems present (32). In advanced-stage mesothelioma patients only very limited tumor specific cytotoxic T-cell responses could be detected (95). This hampered anti-tumor immune response is likely to be caused by the presence of immunosuppressive cells (118).

This may explain, as we reviewed recently, the low number of responders to immune checkpoint antibodies in these patients (29), as for the efficacy of these antibodies, cytotoxic T cell activity is crucial. However, as we did find a large variation in the number of MDSC and also CD8 positive cells in patients with advanced disease NSCLC (32), this also clarifies why responders do occur. In individual patients with lung cancer and mesothelioma, also in the same stage of the disease, a different profile of immunosuppression is present (data submitted for publication).

But still in most patients with advanced disease an *ex-vivo* immune activation is obligatory. As described above, our department has experience with dendritic cell based therapy as cellular immunotherapy (95,117). Combining this therapy with one of the other forms was found synergistic in our animal model (35-37). The results of the patient study on regulatory T cell depletion in combination with dendritic cell immunotherapy are pending.

Immunotherapy in lung cancer and mesothelioma: the way to go

The complex interplay between the tumor and the immune system which differs per patient and per time point causes the need for personalized immunotherapy. Depending on the state of the immune system, different forms of immunotherapy will have to be deployed. Future research will have to take this complex interplay into account.

Another key issue is the definition of effectiveness in clinical trials. Immunotherapeutic approaches show clinical success that may take months to develop, even after an initial period of presumed tumor growth (125,126). Therefore new criteria for development of these drugs in early phase clinical trials are proposed to take these unique tumor response kinetics into account (127). Redefining appropriate clinical trial end points in the coming years is essential to determine the efficacy of the different classes of immunotherapy in patients with cancer at an early phase of drug development.

It is desirable that before the start of any immunotherapy trial proper biomarkers are selected. To make bigger steps these must also be incorporated in early phase trials. Unfortunately large phase III studies have been performed without taking these provisos into account. The recent negative trials on talactoferrin and anti-MUC-1 are probably examples of this absent patient selection beforehand. Unfortunately also at present large phase III trials are enrolling patients without proper patient selection or selection done on unclear conditions. The ongoing phase III trials on immune checkpoint inhibitors purely in squamous cell lung cancer are examples of patient selection without a clear theoretical background but driven on the (unexpected and unexplained) very preliminary data on efficacy.

Conclusions

Immunotherapy is one of the most exciting and promising developments in recent years in the treatment of cancer. Facing the difficulty of its working mechanism and the read-out of the efficacy, investigators have the responsibility to search for biomarkers and outcome parameters, which then have to be embraced by the scientific community.

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