Review Article

The role of the immune system in non-small cell lung carcinoma and potential for therapeutic intervention

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Abstract: Over a hundred years after the first description of this disease, lung cancer represents one of the major challenges in oncology. Radical treatment cannot be introduced in more than 70% of cases and overall survival rate does not exceed 15%. The immunosurveillance of lung cancer may be effective in early oncogenesis but is inhibited in the course of developing a clinically detectable tumor. Very low and heterogenous antigenicity of lung cancer cells leads to passive escape from anti-cancer immune defense. The cytotoxic lymphocytes (CTLs) that play a main role in the anticancer response are actively suppressed in the tumor environment and following regulatory mechanisms inhibit the recognition of tumor antigens by antigen presenting cells. The population of regulatory T cells (Tregs) is augmented and the expression of transcription factor—Foxp3 is markedly increased on tumor cells and tumor infiltrating lymphocytes (TIL). It is accomplished by M2 macrophage polarization, the activity of myeloid derived suppressor cells (MDSCs) and a significantly elevated concentration of cytokines: transforming growth factor beta (TGFβ) and IL-10 in the tumor microenvironment. Very active suppression of immune protection is the predominant role of the programmed death 1 (PD-1)-PD-L1 pathway. The blockage of this pathway was found to be an effective treatment approach; therefore the monoclonal antibodies are being intensively investigated in lung cancer patients. Cytotoxic T lymphocyte antigen-4 (CTLA-4) is the molecule capable of inhibiting the activation signal. The antibody anti-CTLA-4 improves CTLs function in solid tumors and lung cancer patients may benefit from use of this agent. The second way in lung cancer immunotherapy is production of anti-cancer vaccines using recognized cancer antigens: MAGE-A3, membrane associated glycoprotein (MUC-1), and EGF. It was recently shown in ongoing clinical trials that combined therapies: immune- and chemotherapy, radiotherapy or targeted therapy seem to be effective. Immunotherapy in lung cancer has an individual character—there is a need to assess the patient's immune status prior to implementation of immunomodulating therapy.

Keywords: Lung cancer; immune response; cytotoxic cells; regulation; immunotherapy; vaccines

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Introduction

Two years ago the 100-year anniversary of lung cancer was “celebrated”. 100 years after the first description of 374 cases of lung cancer (1) as high as 1.6 million new cases are diagnosed yearly (2,3). In recent years the clinical and biological patterns of lung cancer have been changed and are varying continuously. The increasing incidence of adenocarcinoma, decrease in the proportion of small cell lung cancer and new observations on lung cancer in nonsmokers are the most striking features of this change and remain a challenge for the progress in thoracic oncology. However, an unchanging fact is that lung cancer is the main oncological problem worldwide and it is a leading cause of cancer death among patients with malignancy. The resection rate that is a treatment of choice in early—stage non-small cell types—is as low as 25-30% (3,4).
In the advanced stages of non-small cell lung carcinoma (NSCLC) other therapies are used with some effectiveness: chemotherapy, radiotherapy and biological treatment. Recently it became important to perform appropriate cancer molecular characterization and to select patients individually to a treatment strategy with thorough analysis of the histological type, molecular pattern and evaluation of predictive factors. In practice this molecular characterization is performed by analysis of activating mutations of epidermal growth factor receptor (EGFR) (in clinical practice in exons 19 and 21) and detection of an anaplastic lymphoma kinase (ALK) rearrangement (5). For tumors with activating EGFR mutations, first-line treatment is indicated with an EGFR tyrosine kinase inhibitor (EGFR-TKI, such as geftinib, erlotinib, and afatinib). Anti-EGFR antibody- cetuximab is accepted in some countries as a biological therapy. The treatment with crizotinib is advised for ALK-positive lung cancer (5-7). However, the prevalence of an EGFR mutation in adenocarcinoma of European patients is close to 10%, while in Asian and Japanese patients is up to 30-50% (8). More lung cancer prognostic markers are being published, but without promising effectiveness in practice (5).

Among NSCLC subtypes adenocarcinoma is the most heterogeneous tumor, with known aggressiveness of certain subtypes (i.e., solid tumor with mucus production), and response to anti-EGFR targeted therapy in tumors harbouring EGFR mutations (9,10). This direction of targeted therapy has brought some good results, but only in the appropriate selected patients groups (5). Only a relatively small proportion of patients in our country harbor EGFR mutations so only small numbers of patients benefit from currently available targeted therapies (11). The current therapeutic approach develops in another direction—with taking into account an advantage of the recognition of the immune response in solid tumors. The goal of such new therapies is to support the host’s own anticancer immune response. Here a description of the immune alterations in the course of NSCLC with possible implications for therapy is presented.

**Background to the considerations**

The morbidity due to lung cancer is strongly correlated to age with the greatest risk in the oldest patients groups of both sexes. Age distribution at lung cancer diagnosis is estimated at approximately 6% in patients below 50 years of age, 29% in patients of 60–69 years old, and 44% in patients over 70 years of age (3). In this context the role of immune system senescence has to be revealed. The following alterations characterize an immune-aging (inflamm-aging): shortening of telomeres, histone acetylation and reduction of antiaging molecules such as histone deacetylases and sirtuins, apoptosis, increased concentration of proinflammatory cytokine- IL-6, and Th2 polarization (12). These disorders are inhibitors of anti-cancer immune response in the course of lung cancer. Immuno-senesce enhances the failure of anti-cancer response.

Cigarette smoking is the main risk factor for lung cancer (2,3). The influence of tobacco smoke on lung homeostasis is complex with a predominant feature being suppression of the immune system (13,14). We have previously reported the noxious influence of tobacco smoke on lung immune status (15-17). Apart from tobacco smoke, many other environmental agents permanently affect the lung milieu: dust, allergens and microbes, with resulting oxidative stress and hypoxia. These factors are capable of causing serious modification of lung immune status. For better understanding of the nature of immune disturbances, the continuous process of self- and down-regulation of the function of immune cells cannot be neglected.

The lung immune system has multiple parts: it consists not only of large numbers of immune cells with a complex cytokine network, but also of structural elements of different function, i.e., epithelial, endothelial and mesenchymal cells. In normal conditions an integration of these elements is fixed and the proportion of immune cells rests within a normal range. In my opinion a valuable way for evaluation of the lung immune status, in steady state and during disease, is bronchoalveolar lavage (BAL) fluid examination. BAL analysis is a low-invasive method and the BAL components reflect the local immune response in a large part of the lung. In clinical practice the main indication for BAL analysis is a diagnosis of diffuse parenchymal lung disorders, interstitial lung diseases and infections. In lung cancer the role of BAL in peripheral tumor diagnosis has also been documented (18). This is a quantitative method, already well standardized (19-21). For the lavage, 200 mL of saline is used; the total cell count and differential cell count are determined in the recovered fluid. The referenced BAL pattern in nonsmokers contains total cell count of about 10 million cells, cell viability is more than 90%, the percentage of macrophages >80%, lymphocytes <15%, neutrophils <5%, eosinophils <1% (19,21,22). The effectiveness of BAL in the evaluation of lung immune status in the course of lung cancer has been described in our earlier works (17,23-25). The elements of the immune response in lung cancer patients may...
serve as biomarkers and predictive factors in regards to immunotherapy, applied in clinical practice. BAL may be performed during bronchofiberoscopy, which is an inherent step in the diagnostic procedure. It should be mentioned that knowledge of defense mechanisms in lung cancer is rather limited to data obtained from peripheral blood samples, reflecting the systemic immune response. As concerns local immune response evaluation it is usually performed by the examination of resected tumors. Since the resection rate of NSCLC does not exceed 30% and small cell types are not-resectable per se, in the majority of lung cancer cases the local immune status cannot be studied. From this perspective the BAL analysis is important as it can be performed at any time of the disease, including advanced lung cancer stages.

Is the histologic type of NSCLC important? Today’s classification of NSCLC recommends clear distinction of squamous cell type and adenocarcinoma, which is important in guiding to current treatment with new methods of targeted therapy. In this context it is often important to detect thyroid transcription factor (TTF-1) positive cancer cells as an indicator of glandular differentiation in those cases where it cannot be seen morphologically. TTF-1 is essential for morphogenesis and differentiation of the lungs and is a marker of lung adenocarcinoma. In some studies TTF1 expressing tumors were suggested to be associated with longer survival (26,27). However, the heterogeneity of lung cancer occurs with mixed types entity. Moreover quite often the non-otherwise specified (NOS) type is being diagnosed. In these cases detection of TTF1 positive cells is suggestive for adenocarcinomatous differentiatation. In the daily practice we observed that the EGFR mutation initially restricted to adenocarcinoma is present also in the squamous cell type. Perhaps in the future the molecular classification of NSCLC may be more relevant than the histological one.

Last but not least, cancer stem cells (CSCs) are a new potential target for solid tumor therapy, including a lung cancer therapy (28-30). The phenotype of CSCs is currently widely investigated, the marker CD133/EPCAM being suggested (31,32).

**Cytotoxic attack**

Lymphocytes, macrophages and granulocytes are involved in the anti-cancer battle. The niche of lymphocytes is known as tumor infiltrating lymphocytes (TIL) (33,34), of macrophages—the tumor associated macrophages (TAM) (35,36), of neutrophils—the tumor associated neutrophils (TAN) (37), and of eosinophils—the tumor associated tissue eosinophilia (TATE) (38). The main cell population with activity in anti-cancer immune response is the population of cytotoxic T lymphocytes (CTLs) (39). The CTLs population is represented by CD8+ lymphocytes, CD4+ lymphocytes, natural killer cells (NK), natural killer T cells (NKT) and lymphocytes B (40,41). Cancer cells are killed by induction of apoptosis by cytolotic reaction or membrane-receptor induction of programmed death. The successful cytotoxic attack needs an effective antigen presentation by tumor cells and antigen presenting cells (APC). This is achieved mainly by macrophages and dendritic cells (DCs) (42). The latter migrate to lymph nodes after contact with cancer antigens and activate effector cells by presenting the antigen. A crucial role in APC-lymphocyte signal transmission is played by co-stimulating molecules on APC and related receptors on lymphocyte (Figure 1) (39,43). As cytotoxic CD8+ lymphocytes and CD4+ cells are “soldiers” of the CTL army, the signal pathway B7-CD28 is widely investigated (39). The blockage of APC-CTL action is observed in malignancy and provides the CTLs inactivation.

**Impaired function of the immune system—the mechanisms of immune tolerance**

It is well documented that anti-cancer defense is ineffective in clinically detectable cancers and that the greater is the size of a solid tumor mass, the less effective anti-cancer response is observed (44). Lung cancer cells hide against cytotoxic attack by low antigen presentation and low co-stimulatory molecule expression. Moreover, the lung cancer antigens are unstable and badly defined as a result of multiple genetic and epigenetic alterations during oncogenesis (45). Altogether, it leads to a passive cancer cells escape from immunosurveillance. On the other hand, many other elements of this escape relate to active regulation and suppression of the immune anti-cancer response.

There are many mechanisms of CTL inhibition (Figure 2). An interaction of programmed death receptors on lymphocytes with their ligands on tumor cells leads to apoptosis of lymphocytes. Recently it was revealed that the expression of the programmed death-1 (PD-1) molecule on T cells plays an important role in the context of cytotoxic effect inhibition (46). PD-1 is present on T helper, T cytotoxic, T regulatory cells, B lymphocytes and NK cells. Tumor cells express high levels of PD-1 ligands: B7-H1 (PD-L1) (CD274) and PD-L2 (CD273, B7-DC). The PD-1-PD-L interaction...
has a strong immunosuppressive effect. It has been applied to therapy with the blockade of PD-1/PD-L pathway using a fully humanized PD-1 or PD-L1 antagonistic monoclonal antibodies shown to increase the number and functionality of tumor-specific T cells (39,47-49).

We have previously reported the increased expression of Fas receptor on lymphocytes in the course of lung cancer (50). An interaction of Fas-Fas ligand (Fas-L) causes the death of Fas bearing cells. Apart from an elevated proportion of Fas positive lymphocytes and the high...
expression of Fas receptor on CTLs, the expression of Fas-L on cancer cells is known to be markedly elevated. Alterations of the concentration of soluble forms sFas and sFasL which moderate apoptosis have been also found in NSCLC (51). Thus this receptor pathway plays an important role in the process of reduction of CTL number. To date no therapy targeting this mechanism is currently in clinical evaluation.

Another mechanism of impaired anticancer defense and hiding cancer cells from CTLs attack is a modification of co-stimulatory molecules on cancer cells and on APCs. T cells are the main cytotoxic population that recognizes target cells by interaction with APCs. B7 molecule (CD80/CD86) on the APC and the CD28 receptor on lymphocyte are necessary to activate the cytotoxic effect. However, B7 molecules are also capable of sending a suppressive signal by association with CTLA4 (Cytotoxic T cell antigen 4) (52-54). CTLA4 is a molecule capable of inhibiting the TCR signal on T cells having homology with the CD28-co-stimulatory molecule with strong affinity. CTLA4 leads to inhibition of cell cycle progression, decreased release of IL-2 and increased transforming growth factor beta (TGFβ) production by blocking CD28. By connection with forkehead box P3 (Foxp3), CTLA4 is constitutively expressed on regulatory T cells (Tregs) and promotes their regulatory function (55-57). There are two forms of CTLA-4 expression: on the cell surface after activation, and intracellularly as storage (58,59). Our study (data unpublished) showed a difference in the CTLA-4 expression on T cells deriving from peripheral blood (PB) of lung cancer patients and healthy subjects. The CTLA-4 surface expression in cancer patients was significantly higher, while the intracellular domain was decreased in PB of cancer patients compared to healthy subjects. Our results indicate the importance of cellular traffic of this molecule in malignancy.

**Therapeutic approach I**

PD-1 and CTLA-4 are considered the main checkpoint molecules for effective immunotherapy in solid tumors. PD-1 antagonists are presented by PD-1 or PD-L1 antibodies: nivolumab, lambrolizumab and pidilizumab. The results of recently ongoing trials with anti-PD-1 antibodies are promising, although the association with detection of PD-L1 on tumor cells before treatment is controversial (39,60).

The anti CTLA-4 IgG1 humanized antibody—ipilimumab binds to CTLA-4 and prevents the inhibition of CD28/B7 signaling. It leads to T cell activation and depletion of Tregs. Similarly to anti-PD-1 agents, the anti-CTLA-4 antibody has shown some benefits, particularly in combination with chemotherapy (48).

Recent studies confirm the importance of regulatory cells in the modification of immune response in malignancy. Regulatory T lymphocytes (Treg) are capable of inhibiting the function of CD4+ and CD8+ lymphocytes, dendritic cells and NK cells (55,61,62). Treg cells play an important role in the immune surveillance and tolerance. The source of natural Tregs (nTregs) is the thymus. The second source is a population activated peripherally (induced Tregs, iTregs). The suppressing cytokines: interleukin-10 (IL-10) and TGFβ are involved in the peripheral activation of Tregs (63). In the lung cancer milieu the concentrations of IL-10 and TGFβ is high, and these cytokines are secreted by cancer cells and immune cells stimulated by cancer (64). They constitute an active regulation of immune response by cancer through induction of Tregs. Tregs are identified by expression of the panel of antigens: a Foxp3, CD25, glucocorticoid-induced TNF-receptor (GITR) (CD357), lymphocyte-activation gene 3 (LAG3), cytotoxic T lymphocyte antigen-4 (CTLA4) and CD127. The Tregs are defined by expression of CD4, CD25, Foxp3 and low CD127 (65,66). Foxp3 is a transcription factor necessary to keep a proper T reg function. An increased expression of Foxp3 was found in the cancer cells and in TILs and the presence of Foxp3 in breast cancer as well as in lung cancer was a negative prognostic factor (65,67-71).

In addition to type Th1 and Th2 cells, the concurrent polarisation direction of T cells is Th17 differentiation. It is not so pronounced as Tregs, but regarded as significant in regulation of immune response in malignancy. These pluripotent cells are active in antimicrobial defense, albeit their proliferative and cytotoxic effect is low. Th17 cells are defined by production of IL-17A. Other cytokines play a role in Th17 differentiation, i.e., IL-6, IL-1β and IL-23. It is presumed that IL-6 inhibits Tregs development with stimulation of Th17 (72,73). This example of the plasticity of immune system is accomplished by known TGFβ function: TGFβ in low concentration induces Th17 differentiation, while in high concentrations induces Tregs Foxp3+ maturation (73). To our knowledge, there is no direct data on the anticancer effect of Th17. Until now some results indicate that the effect of Th17 is complex as the IL-17 action in cancer milieu is pleiotropic: suppressive and stimulating. The stimulating effect is related to proangiogenic role of IL17A (74-76).
**Therapeutic approach II**

The depletion of Tregs by anti-CD25 antibody was proven to be ineffective (77). More rational is putting efforts to change the polarization of T cell by enzymatic and cytokine profile modification to achieve a re-polarization of Tregs to Th profile. Complex engineering by using the indoleamine 2,3-dioxygenase (IDO) inhibitor plus vaccine provided such a re-polarization (77).

Alveolar macrophages play an important role in lung cancer defense (35). In the solid tumors a population of TAM was widely investigated and their relation with cancer cells is complex. Generally, the function of TAM population is impaired, but their regulatory function in lung cancer immunity is postulated (78). Traditionally, macrophages were considered to be a uniform cell population, but recently have been divided to different phenotypes: M1, M2 and macrophages with regulatory properties (79-81). M1 macrophages as effector cells play an immunostimulating role by secretion of cytokines (IL-12 among others) and reveal phagocytic properties. M2 macrophages with their suppressive function are the main constituents of TAM population, promoting angiogenesis and wound healing (80). They release mainly IL-10. M1 and M2 are activated by different ways: M1 by LPS and IFNγ, while M2 by IL-4, IL-10, IL-13 and TGFβ. Such different polarization of macrophages is detected by diverse phenotype, i.e., M1 cells express mainly CD40, while M2 express CD163, as we have recently confirmed by immunocytochemistry staining (82). For regulatory macrophages no defined surface antigenicity was found, therefore identification is based on cytokine production (TGFβ and IL10). Further subtyping of the M2 population has been recently proposed on the basis of the inductors and mediators balance (83). The presence of M1 in cancer milieu is favorable (84), however M2 vastly predominate among TAM. The potential shift of M1-M2 was confirmed in our experiments by immunocytochemical staining (82).

Myeloid derived suppressor cells (MDSCs) originate as bone marrow derived hematopoietic cells and precursors of immune cells other than lymphocytes. An augmentation of circulating MDSCs in serious diseases and in malignancy has been documented (85). The MDSCs identification can be done by detection of antigens: CD11b, CD14, CD13, HLADR (85). The mediators secreting by cancer cells (i.e., GM-CSF, IL-6 and IL-1) are essential to MDSCs survival in the tumor microenvironment. MDSCs are able to inhibit T cells activation and DC differentiation, and to promote Tregs. Since arginine, cysteine and nitric oxide (NO) are necessary for a proper T cell activation and memory type differentiation, MDSCs inhibit immune response by competitive use of these substrates (86). MDSCs produce a number of radical species and suppressor cytokines, and by this way favour angiogenesis, vasculogenesis and metastases (39,87,88). The process of epithelial-mesenchymal transition (EMT) plays an important role in the context of MDSCs function and inflammatory cell migration. Until now some signaling pathways of cell to cell contact, cell polarity and cell- matrix modulation have been recognized however, the process is complex (89,90).

**Therapeutic approach III**

Efficacy of 5, 6-dimethylxanthenone-4-acetic acid (DMXAA, Vadimezan) for activation of the antitumor properties of TAM was described in an animal model by Fridlender et al. (91,92). Reduction of M2 and MDSCs function may be achieved by blocking the immunosuppressive enzymes and by reversing the hypoxia status in the tumor microenvironment (35,36,93). Nitroaspirin and sindelafil were found to be effective blockers of arginase and NO synthase, enhancing an effectiveness of anticancer vaccines (77). The anti IL-10 and anti-CD40 antibodies combined with chemotherapy were associated with the change of macrophage profile (94). Some unpecific substances are also capable of inhibiting MDSCs (39).

Cancer cells release many suppressor cytokines. In this context TGFβ is the best-recognized compound. An increasing concentration of TGFβ in cancer tissue and in the cancer cells culture as well as in the cancer milieu has been reported (15). The complex TGFβ function and role in tumor progression are presented in Figure 3. Several interleukins reveal similar immunosuppressive effect in the lung cancer environment, including IL-10 and IL-2. The latter induces CTLA4 and mediators: vascular endothelial growth factor (VEGF), prostaglandin E2, arginase, reactive oxygen species, sFas, sFasL (39,95).

Regarding the complex role of TGFβ, it is unlikely that a use of a simple anti-TGFβ agent will be effective in cancer immunotherapy. Thus TGFβ is used only as an adjuvant in anti-cancer vaccines production and in combination with other therapies used for CTLs stimulation (39,96,97). Sometimes TGFβ promotes positive immune response and stimulates the CTLs, suggesting a pluripotent function of the cytokine (98). For example the interesting experimental study showed the different effect of TGFβ in relation to the
time of tumor development: injection of anti-TGFβ agent before the injection of cancer cells resulted in inhibition of the active CTLs. Thus it may indicate a positive role of TGFβ in anticancer defense in the initial, pre-clinical stage of malignant disease (96).

### Tumor antigens and vaccines production

There are two well-known lung cancer antigens that are used for vaccine production (99). The Melanoma Associated Antigen (MAGE-A3), absent on normal cells, is detected on NSCLC cells in about 35-50%, the majority being of squamous histological type (100). The presence of MAGE-A3 is associated with advanced stages of cancer. Some epitopes of this antigen are well recognized by HLA-I restricted lymphocytes Tc and these properties are used for vaccines production. Membrane associated glycoprotein (MUC-1) is associated with epithelial and glandular malignant tissue and is often overexpressed on cancer cells. A high MUC-1 expression is associated with lung cancer cell migration, resistance to apoptosis, and resistance to chemotherapeutic agents (101). The superficial domain of MUC-1 depending on the status of glycosylation is highly immunogenic; it makes possible the use of MUC-1 for T cell response stimulation (102). Recently another transmembrane glycoprotein-epithelial cell adhesion molecule (EpCAM) has been widely investigated in lung cancer; it was found that the detection of circulating lung cancer cells with EpCAM/MUC-1 overexpression was associated with poor prognosis after curative surgery (103).

There are numerous new neo-antigens recognized by genome sequencing of KRAS, EGFR, and ALK. The antigens and proteins encoded by these genes are present on lung cancer cells. The point mutations of these antigens make them immunogenic and useful for vaccine production (44,104).

The anti-cancer vaccines have been extensively investigated since the 1990s. The idea of vaccine production is to enhance antigen presentation by educated DCs. The vaccine formulation comprises the immunogenic tumor-associated antigens formed as peptides, recombinant proteins, gangliosides or whole tumor cells, which are combined with an adjuvant prior to potentiate the immune response (105). This immunoadjuvant is a viral vector, dendritic cell or liposome formulation. The examples of vaccines used in therapeutic approach in lung cancer are presented in the Table 1.

The results of recently conducted trials showed that anti-lung cancer vaccines failed to meet expectations with only some benefits in a selected group of patients (106). Therefore an effective direction in studies maybe individualization of immune treatment: the detection of cancer antigen before vaccination (MAGE-A3), enumeration of cytotoxic cells (anti-MUC-1 vaccine was shown to be effective in patients with normal number of activated NK cells) or individual production of dendritic cells with control of patient immune status (107). For evaluation of immunotherapy results the new criteria beyond RECIST WHO are needed and were recently described by Wolchok et al. on the basis of melanoma immunomodulating treatment (108). The immune-related response criteria (irRC) were introduced and the main consideration is that immunotherapy could be continued even in the case of radiological pattern of tumor progression.

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**Figure 3** Role of transforming growth factor beta (TGFβ) in lung cancer progression. CTLs, cytotoxic lymphocytes; Foxp3, forkhead box P3.
<table>
<thead>
<tr>
<th>Immunotherapy</th>
<th>Immune target</th>
<th>Composition/mechanism of action</th>
<th>Clinical trial/Patients NO/Stage, criteria/Intervention</th>
<th>Results</th>
</tr>
</thead>
</table>
| Ipilimumab    | CTLA-4        | Monoclonal antibody            | (I) Phase II/204/Chemotherapy naïve advanced NSCLC/Carboplatin-paclitaxel with either placebo, concurrent ipilimumab or phased ipilimumab to maintenance ipilimumab or placebo every 12 weeks  
   (II) Phase III/920/IV squamous type recurrent/Carboplatin–paclitaxel with either placebo, concurrent ipilimumab or phased ipilimumab to maintenance ipilimumab or placebo every 12 weeks  
   (III) Phase III/1100/Chemotherapy naïve advanced disease/Carboplatin-paclitaxel with either placebo, concurrent ipilimumab or phased ipilimumab to maintenance ipilimumab or placebo every 12 weeks | Immune related PFS better for ipilimumab + chemotherapy |
| BMS-936558 nivolumab | PD-1 | Monoclonal antibody | (I) Phase I with expansion/122/After completion chemotherapy/Dose-escalation study with nivolumab: 1, 3 or 10 mg/kg  
   (II) Phase III/264/Squamous cell NSCLC recurrent or progressing during/After platinum-based chemotherapy for stage IIIB/IV nivolumab vs. docetaxel  
   (III) Phase III/574/Nonsquamous cell NSCLC recurrent or progressing during/After platinum-based chemotherapy for stage IIIB/IV nivolumab vs. docetaxel | OR in 33% patients with squamous NSCLC |
| Pembrolizumab | PD-1 | Monoclonal antibody | (I) Phase II/III /300/Previously treated PD-L1-positive/NSCLC, pembrolizumab vs. docetaxel | Pending |
| Pidilizumab | PD-L1 | Monoclonal antibody | PD-L1-positive locally advanced or metastatic NSCLC, after platinum failure | Pending |
| Talactoferin | Nonspecific | Recombinant form of lactoferin | IIIB/IV combination with first line chemotherapy | RR 47%, OS—6.1 mos (better than placebo) |

Table 1 (continued)
<table>
<thead>
<tr>
<th>Immunotherapy</th>
<th>Immune target</th>
<th>Composition/mecanism of action</th>
<th>Clinical trial/Patients NO/Stage, criteria/Intervention</th>
<th>Results</th>
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<tbody>
<tr>
<td>Vaccines</td>
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<tr>
<td>TG4010</td>
<td>MUC1</td>
<td>MUC-1 antigen-specific liposomal vaccine with IL-2 gene</td>
<td>(I) TIME trial, phase IIB/III, randomized, placebo controlled /1000/ Treatment-naive MUC1+, stage IV NSCLC/TG4010 plus chemotherapy vs. placebo plus chemotherapy</td>
<td>Some benefit for patients with normal number of activated NK cells</td>
</tr>
<tr>
<td>BLP-25</td>
<td>MUC1</td>
<td>MUC-1 antigen-specific liposomal vaccine</td>
<td>(I) Phase II/171/After first line chemotherapy/Vaccine + BSC vs. BSC (II) Start phase III randomized/1514/Stage III NSCLC after chemoradiotherapy/Vaccine vs. placebo (III) Inspire phase III randomized/420/Asian patients stage III NSCLC after chemoradiotherapy/Vaccine vs. placebo</td>
<td>3-y OS 31% for BLP-25 vs. 17% BSC</td>
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<tr>
<td>CIMavax</td>
<td>EGF</td>
<td>Recombinant EGF</td>
<td>(I) Phase II/80/After first line chemother/ Cyclophosfamid + vaccine + BSC vs. BSC (II) Phase III/438/After first line chemother/ Cyclophosfamid + vaccine + BSC vs. BSC</td>
<td>Difference significant in patients &lt;60 years</td>
</tr>
<tr>
<td>MAGE-A3</td>
<td>MAGE-A3</td>
<td>Recombinant MAGE-A3 + protein D Haemophilus influenzae + adjuvant</td>
<td>(I) Magrit trial, phase III /2270/Completely resected plus stage IB, II or IIIA NSCLC (cohorts with or without adjuvant chemotherapy)/ Vaccine vs. placebo</td>
<td>Some benefit, depended on gene signature</td>
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<tr>
<td>Belagenpumatucel Lucanix®</td>
<td>TGFβ2</td>
<td>Irradiated NSCLC cell lines transfected with a plasmid containing the TGF-β2 antisense transgene</td>
<td>(I) Stop phase III randomized/506/III, IV after platinum based chemotherapy/ BSC + vaccine vs. BSC + placebo</td>
<td>OS dose depended-better for higher dose</td>
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NO, number; NSCLC, non-small cell lung carcinoma; PFS, progression-free survival; OR, objective response; BSC, best supportive care; OS, overall survival. TGFβ2, transforming growth factor beta; MAGE-A3, Melanoma Associated Antigen.
The immune response in lung cancer is complex, hence the immunotherapy should be multivalent in combination with other therapeutic options. The most current promising direction is to combine immunotherapy with a conventional chemo- and radiotherapy. The rationale for such combination is manifold: by induction of immunogenic cell stress and cell death the cytotoxic agents are capable of enhancing tumor antigenicity, likewise radiotherapy can induce antigen expression and modulate antigenic repertoire (44). The regulatory/suppressor cells (Tregs, M2, MDSCs), an actively multiplied population, seem to be more susceptible to chemotherapy than the less numerous CTLs. Some cytotoxic agents have been shown to kill myeloid suppressor cells and inhibit FoxP3 expression, leading to reduction of the number of Tregs. Radiotherapy favors the release of proinflammatory cytokines, promotes antigen cross-presentation, recruits immune cells, supports DCs migration to lymph nodes and induces death cell receptors on tumor cells (39,44). The immunomodulatory properties of targeted therapy (e.g., cetuximab, crizotinib) have also been described (44,109,110). These observations are currently applied in clinical trials (111).

**Figure 4** summarizes today’s goals of immunomodulating therapies in lung cancer. Almost every day delivers data on new therapeutical trials providing hopeful results in our battle against this tumor. Some limitation of the potential success of immunotherapy is due to the large number of advanced stages of NSCLC in time of the diagnosis and the fact that this kind of treatment is restricted to these stages in current clinical trials. However, the evidence of some benefit of complex treatment with immunotherapy as an additional arm with chemo-radiotherapy gives us hope for the future.

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**References**


37. Fridlender ZG, Albelda SM. Tumor-associated


96. Quatromoni JG, Suzuki E, Okusanya O, et al. The timing...
of TGF-β inhibition affects the generation of antigen-specific CD8+ T cells. BMC Immunol 2013;14:30.


