

The Non–Small Cell Lung Cancer Immune Contexture A Major Determinant of Tumor Characteristics and Patient Outcome

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Abstract

Solid tumors, beyond mere accumulation of cancer cells, form a complex ecosystem consisting of normal epithelial cells, fibroblasts, blood and lymphatic vessels, structural components, and infiltrating hematopoietic cells including myeloid and lymphoid elements that impact tumor growth, tumor spreading, and clinical outcome. The composition of the immune microenvironment is diverse, including various populations of T cells, B cells, dendritic cells, natural killer cells, myeloid-derived suppressor cells, neutrophils, or macrophages. The immune contexture describes the density, location, and organization of these immune cells

within solid tumors. In lung cancer, which is the deadliest type of cancer, and particularly in non–small cell lung cancer, its most prevalent form, reports have described some of the interactions between the tumor and the host. These data, in addition to articles on various types of tumors, provide a greater understanding of the tumor–host microenvironment interaction and stimulate the development of prognostic and predictive biomarkers, the identification of novel target antigens for therapeutic intervention, and the implementation of tools for long-term management of patients with cancer.

Keywords: non–small cell lung cancer; tumor immunology; immunotherapy; prognosis

Worldwide, lung cancer is the most common cause of cancer-related deaths (1). There are several histological subtypes of lung cancer, which differ in their relative frequency, location within the lung, and tendency to metastasize. Small cell lung cancer (SCLC), which often presents with widespread disease at the time of diagnosis, represents about 15% of all lung cancer cases (2). Non–small cell lung cancer (NSCLC) represents the remaining 85% of lung cancers, and the 5-year overall survival (OS) rate is about 15% (3). At present, NSCLC staging is based on the seventh

tumor/lymph node/metastasis (TNM) classification (4, 5). This classification provides a standardized description of the disease and describes the severity of cancer based on the size and/or extent of the primary tumor, and whether or not it has spread to lymph nodes and/or distant organs. TNM stage grouping is currently considered the best determinant of the prognosis of patients with NSCLC (6, 7).

We have witnessed a change of view of cancer as an autonomous cellular disease comprising six biological capabilities (8) to that of a regulated disease involving

the immune components of their microenvironment, called *immune contexture*. To their original hallmarks of cancer published in 2000 (8), Hanahan and Weinberg added two emerging hallmarks and two enabling characteristics, including “evading immune destruction” and “tumor-promoting inflammation” (9). This major evolution in the view of neoplastic disease reflects that cancer is now considered a complex ecosystem with many players that could impact tumor growth, spread, and clinical outcome (10, 11). During cancer development, tumor cells interact

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with their microenvironment and the immune contexture shapes their eventual destiny. Increasing evidence has established the potential key contribution of tumor-infiltrating immune cells in the development and progression of cancers and in the tumor response to therapy (12, 13). In colorectal cancer, a strong association between the quality, quantity, and coordination of intratumoral immune cell infiltration and patient survival has been elegantly reported (14, 15). Strikingly, the type and density of T cells infiltrating these tumors were found to be more powerful prognostic factors than standard pathological criteria, underscoring the need for clinicians to consider infiltrating immune cells when determining the prognosis of patients with cancer (16). Moreover, the organization of the immune cells and more particularly the presence of local lymph node–like structures seem to be of major importance in shaping protective antitumor immune responses (17–19).

There is now growing evidence that innate and adaptive immune cells interact in the lung tumor microenvironment and that the immune contexture of these events (i.e., the structural and functional links between local immunity and other variables in the tumor microenvironment) impacts cancer cells and clinical outcome. Here, we review the association between NSCLC and immune surveillance, immune escape, and immune subversion mechanisms as well as the prognostic value of the tumor immune microenvironment.

Immunosurveillance of Lung Cancer

General Concept

The immune-editing concept proposed by Dunn and colleagues (20), reflecting the evolution of the interactions between immune and tumor cells during cancer development, can be applied to NSCLC. Although it is difficult to provide proper proof of immunosurveillance in humans (21), a large body of evidence supports the idea that developing cancers are not ignored by the immune system. Evidence for immunosurveillance of lung cancer can be found in patients with HIV infection who have an increased frequency of virally induced malignancies (22, 23), and in immunosuppressed organ transplant recipients who have a higher risk of

developing NSCLC (24). Further support of immunosurveillance in NSCLC can be found in studies that have demonstrated the ability of the immune system to spontaneously recognize tumor-associated antigens (TAAs) (25). In addition, immune-mediated paraneoplastic syndromes are often encountered in lung cancer (26). These syndromes occur in up to 15% of patients with cancer, and lung cancer was found to be the malignancy most frequently associated with paraneoplastic syndrome (27).

Paraneoplastic neurologic disorders develop in patients with cancer when an efficient antitumor immune reaction attacks the nervous system, because targeting antigens expressed by cancer cells share antigenic properties with healthy neurons (28). Autoimmune responses against Hu proteins have been described, and HuD, a Hu family member normally expressed in neurons, is frequently observed as aberrantly expressed in lung cancer cells. There is other evidence that patients with lung cancer may also produce antibodies against other antigens. One example, among others, is found in studies showing the presence of SOX antibodies in the sera of patients with lung cancer with and without paraneoplastic neurologic disorders (29). Together, these results show that lung cancers can be immunogenic tumors eliciting different serum antibody responses. Moreover, as reported for other solid tumors, the immune microenvironment of NSCLC has a strong prognostic value (12). Altogether, these data support the idea that the immune system is able to recognize and eliminate malignant cells.

Actors in Immunosurveillance

In lung tumors, the immune infiltrate comprises adaptive and innate immune cell populations (12, 30, 31). These immune cells are not randomly distributed within the tumor but rather are highly organized (Figure 1) (18). This high degree of organization is reflected by the presence of ectopic lymph node–like structures or tertiary lymphoid structures (TLSs). As reported for infectious diseases (32), autoimmune disorders (33), and allograft rejection (34), the intratumor ectopic lymphoid structures are composed of a B-cell follicle containing follicular helper T cells, tingible body macrophages and a network of follicular dendritic cells, and a T-cell area with mature dendritic cells

(DCs), and are surrounded by high endothelial venules (Figure 1) (18, 35). Direct evidence of class switching has not been reported yet, but it has been observed that activation-induced cytidine deaminase, an indispensable enzyme in both class switch recombination and somatic hypermutation, is expressed by germinal center B cells of some ectopic lymphoid structures (36). Several integrins, adhesion molecules, and chemokines are likely to play a role in the recruitment of T cells from the blood to TLSs (35). As previously shown in secondary lymphoid organs (37), it has been hypothesized that this process implicates rolling and transmigration via high endothelial venules expressing peripheral node addressin (35). The role of TLSs has been well documented in respiratory immunity during viral infections. Notably, Moyron-Quiroz and colleagues have shown in two elegant studies that mice lacking spleen, lymph nodes, and Peyer's patches were able to mount robust primary but also secondary B- and T-cell responses to inhaled influenza, due to compensatorily developed TLSs (38, 39). A body of evidence suggests that the presence of TLSs impacts on the local immune microenvironment in the lung. First, high densities of TLSs are associated with high numbers of activated and effector-memory CD8⁺ T cells inside the tumor (40). Another argument for a role of TLSs in the antitumor immune reaction is the presence of measurable IgG and/or IgA to tumor antigens from B cells purified from TLS-containing lung tumor tissues (Figure 1). Indeed, Germain and colleagues have reported that among 34 patients with NSCLC tested, more than 40% developed antibody reactivity against up to seven different TAAs (including LAGE-1, melanoma antigen [MAGE] family antigens, TP53, and NY-ESO-1) (36). In lung cancer, the positive correlation between high densities of TLSs and improved survival of patients supports the idea that these ectopic lymphoid structures are important in shaping protective immune responses (18, 36, 40). Interestingly, we found that patients with few TLSs had poor survival despite high numbers of infiltrating CD8⁺ T cells, suggesting that CD8⁺ T cells not educated *in situ* within TLSs are inefficient in controlling tumor progression in early- and late-stage NSCLC treated or not with neoadjuvant chemotherapy (40; R. Remark

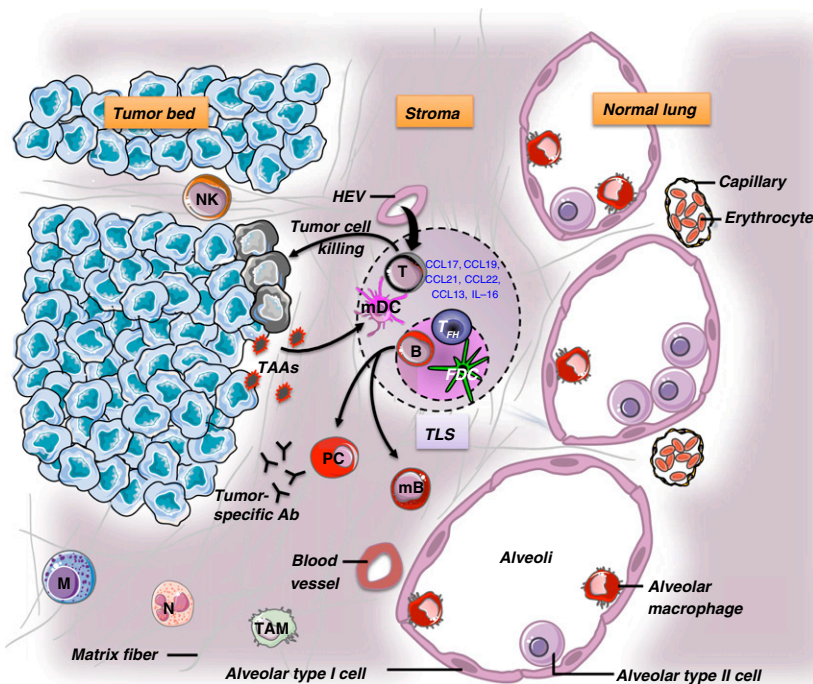


Figure 1. The immune contexture of non-small cell lung cancer (NSCLC). The immune microenvironment of lung tumors is composed of T cells, B cells, natural killer (NK) cells, mature and immature dendritic cells (DCs), tumor-associated macrophages (TAMs), neutrophils, and mast cells. The great majority of immune cells are found at the interface between the tumor and the normal tissue, and some of them are organized in tertiary lymphoid structures (TLSs). The latter are considered a gateway for the entrance of immune cells from the blood to the tumor (via peripheral node addressin-expressing high endothelial venules [HEVs]). This process is highly regulated through chemokine/chemokine receptors, interleukins, integrins, and adhesion molecule expression or secretion. Ab = antibody; B = B cell; FDC = follicular dendritic cell; M = mast cell; mB = memory B cell; mDC = mature dendritic cell; N = neutrophil; PC = plasma cell; T = T cell; TAAs = tumor-associated antigens; T_{FH} = follicular helper T cell.

and D. Damotte, unpublished data). These data suggest that an immune reaction might occur within lung tumors independently of the secondary lymphoid organs. However, further analyses on the potential association between the presence of TLSs and the immunogenicity of tumors, as well as the specificity of the immune response, are needed to know the drivers leading to the formation of such structures and whether they are specific to tumor cells.

Few studies have been performed on the immune contexture in metastases or lymph nodes. Unpublished data from our group revealed that an immune contexture is present and organized in primary lung tumors, liver metastases from lung cancer, and lung metastases of various origins (colorectal, renal cell, and breast cancers, as well as melanoma). We also reported that whereas the immune contexture differs between lung metastases from colorectal cancer and lung metastases from renal cell carcinomas, there was

a significant correlation in the density of immune cell infiltrates between primary and metastatic sites in the same patient, conferring similar prognostic value (41). This comparative analysis of metastases from colorectal and renal cell cancers within the same organ, the lung, in relation to the primary site suggested that the malignant cell rather than the host tissue influences the establishment of a specific immune contexture driving clinical outcome.

Immunosuppressive Cells in the Immune Microenvironment

Myeloid-derived suppressor cells (MDSCs) are important components of the immune suppressive network and can inhibit host protective antitumor immunity (42). By producing vascular endothelial growth factor, basic fibroblast growth factor, hypoxia-induced factor 1, tumor growth factor (TGF)- β , matrix metalloproteinase 9, and generating reactive oxygen species,

MDSCs create a favorable environment for tumor growth and metastasis, as well as neoangiogenesis (42–44). In the lung, these myeloid cells expressing L-arginase and inducible nitric oxide synthase are implicated in the suppression of CD8⁺ T-cell proliferation and decrease CD3 ζ expression (45, 46). MDSC numbers are associated negatively with responsiveness to chemotherapy and positively with shorter survival (45, 46). In a murine model of lung cancer, the targeting of MDSCs enhanced effector and memory CD8⁺ T-cell responses, as well as natural killer (NK) cell and antigen-presenting cell activity (47).

Regulatory T cells (Tregs) are also found in NSCLC and play a role in the control of antitumor immune reactions by the following mechanisms: (1) inducing the expression of B7-H4 by antigen-presenting cells (APCs) (48), (2) promoting the direct killing of T cells and APCs (49, 50), (3) inducing the expression of indoleamine 2,3-dioxygenase by APCs (51), (4) disturbing cell metabolism via the production of adenosine, or (5) secreting IL-10, IL-35, and TGF- β (52). In lung tumors, these cells are likely to play a role in suppressing cytotoxic T-cell responses (30). As suggested in breast cancer, the location of Tregs in the tumor microenvironment seems of major importance in the inhibition of antitumor immune responses (53).

Tumor Intrinsic Factors with Immunosuppressive or Immunogenic Potential

As described in many neoplastic diseases, lung tumor cells develop escape mechanisms. It has been demonstrated that many effector immune cells are anergic and have reduced functions in the lung tumor microenvironment (23, 31). In addition, lung tumor cells may induce a loss or down-regulation of HLA class I molecules during tumor progression, modulating the susceptibility of tumor cells to lysis by cytotoxic CD8⁺ T lymphocytes and NK cells (54). It has also been reported that lung cancer cells are able to produce immunosuppressive factors in the tumor microenvironment. For example, they can secrete a soluble form of MHC class I chain-related molecule A, inducing down-regulation of NKG2D expression of CD8⁺ T cells and NK cells, thereby impairing lysis of cancer cells by these effector cells (55). Tumor cells are also known to secrete immunosuppressive cytokines such as

IL-10 or TGF- β or the tryptophan catabolic enzyme indoleamine 2,3-dioxygenase, which are key players involved in the inhibition of immune responses and enhancing the proliferation of cancer cells (56, 57).

Lung cancer cells, among other components of the tumor microenvironment, express chemokine receptors and produce chemokines that regulate the trafficking of immune and cancer cells. CXCR4, which promotes tumor cell survival, proliferation, invasion, and metastasis, was reported as being overexpressed in tumor cells as compared with normal cells (58). The role of the CXCR4–CXCL12 axis has been highlighted in the metastatic process in NSCLC (59). It has also been suggested that the CCL20–CCR6 axis promotes NSCLC disease progression via their proinflammatory and proliferative effects (60). Finally, CXCR1 and CXCR2 as well as their ligand CXCL8 are expressed and secreted by NSCLC cells (61). CXCL8 could act as a growth factor for tumor cells in an autocrine and/or paracrine manner.

It is also important to note that some genetic alterations have been described in lung cancers (62). The most frequently encountered in lung tumors involve mutations in *KRAS*, *TP53*, *FHIT*, *EGFR*, *CDKN2*, *LKB1*, *RB*, and *MYC* genes. Epigenetic modifications leading to changes in the degree of methylation or acetylation of histone and inducing a modification in gene expression have also been reported, such as hypermethylation of *TP16*, *CDH13*, or adenomatous polyposis coli (63, 64). There is now increasing evidence that oncogenes impact the tumor microenvironment to promote immune escape. For example, Akbay and colleagues have demonstrated in a murine lung tumor model that signaling via mutant epidermal growth factor receptor in tumor cells directly up-regulates tumor programmed cell death ligand 1 (PD-L1) expression (65). It has also been shown that the loss of *Lkb1* and *Pten* leads to lung squamous cell carcinoma with elevated PD-L1 expression (66). In a model of pancreatic cancer, it has been proposed that oncogenic *Kras* restrained the antitumor immune response through the production of granulocyte-macrophage colony-stimulating factor and the subsequent suppression of T-cell immunity (67). In the lung, several tumor antigens have been found: antigens encoded by cancer germline genes or mutated

antigens. The presence of these cancer germline antigens was found to be associated with poor prognosis (68). Yet, antibodies and/or circulating effector T cells can spontaneously develop against alterations observed in NSCLC. For example, at the time of surgery, 7% of patients with NSCLC have circulating serum antibodies to NY-ESO-1 and another 7% have antibodies to TP53 (S. Gnjatic and N. Altorki, unpublished data). The presence of naturally occurring antibody responses to NY-ESO-1 usually correlates with the presence of circulating specific CD4⁺ and CD8⁺ T cells (69). TAAs such as MAGE-A3 have been used to vaccinate patients with NSCLC in the adjuvant setting (70), although unfortunately no clinical benefit was observed in a randomized phase 3 trial. Indeed, this antigen-specific cancer immunotherapeutic (MAGRIT) failed to hit first and second coprimary end points, as it did not significantly extend disease-free survival when compared with placebo.

Immune Checkpoint Expression and Immunotherapeutic Intervention in NSCLC

Several reports have shown that tumors are able to coopt certain immune checkpoint pathways to escape control by the immune system, particularly by T cells that are specific for tumor antigens (71). Indeed, effective antitumor immunity depends at least in part on cytotoxic T lymphocytes, whose fate and killing activity are the results of a balance between positive and negative signals conferred through interactions between various coregulatory receptors and ligands. They include inhibitory molecules such as programmed cell death protein-1 (PD-1), cytotoxic T-lymphocyte-associated protein-4 (CTLA-4), and T-cell immunoglobulin and mucin domain-3 (TIM-3) that can be expressed by T cells, and their ligands (PD-L1, PD-L2, B7-H2, etc.) that are expressed by APCs and cancer cells (71).

In NSCLC, it has been reported that 20–60% of tumors were positive for PD-L1 and/or PD-L2 at lower frequency (72). The expression of PD-L1 and PD-L2 on tumor cells was demonstrated on the cell membrane, in the cytoplasm, or both, in a focal or scattered pattern. The expression levels of both ligands were usually lower on tumor cells than on activated monocytes.

High expression of PD-1 is frequent on activated and exhausted TILs (73). In the lung, high expression of PD-1 on tumor-infiltrating CD8⁺ T cells was associated with impaired T-cell function. Blocking the PD-1/PD-L1 pathway induced increased T-cell proliferation and cytokine production. High PD-L1 tumor expression has been correlated with poor prognosis in some studies (74, 75). However, some studies have reported a lack of association with clinical outcomes or even with improved influx into and survival of lymphocytes in the tumor microenvironment (72). Velcheti and colleagues have shown an association of PD-L1 protein expression with increased TILs and longer survival (76). One explanation among others is that the blockade of PD-1/PD-L1 interactions affects the development, maintenance, and function of PD-1-expressing Tregs (77). CTLA-4 was found on the cell surface and in the cytoplasm of tumor cells in about 50% of patients with NSCLC. The expression pattern was heterogeneous. CTLA-4 overexpression was found to be associated with good survival (78). This unexpected result was explained by the expression of CTLA-4 by tumor cells that can mediate negative signals, comparable with those observed in T cells, and possibly leading to a reduced inflammatory microenvironment.

Because most of the immune checkpoints are initiated by ligand–receptor interactions, they can be blocked or modulated by specific antibodies (79, 80). Ipilimumab, a monoclonal blocking antibody (IgG1) against CTLA-4, was the first of this class of cancer immunotherapeutics to be approved by the U.S. Food and Drug Administration (FDA) and more recently, the FDA also approved pembrolizumab, a monoclonal antibody (IgG4) against PD-1. Although NSCLC has not traditionally been considered an immunogenic disease, a better understanding of immunosurveillance and the identification of new targets of immunomodulation have led to the development of several clinical trials of checkpoint blockade therapies (81) to enhance natural antitumor immunity and induce strong and durable clinical responses in patients with NSCLC (Table 1). Interestingly, it has been suggested that individuals with preexisting antitumor immune responses may be those who are more likely to experience clinical

Table 1. Summary of Clinical Trials Evaluating Various Immune Checkpoint Mediators for Patients with Non–Small Cell Lung Cancer

Target	State of Clinical Development	Trial Design	Conclusions
CTLA-4	Phase 1 trial (NCT02040064)	Open-label, safety, tolerability, and efficacy study of tremelimumab plus gefitinib in EGFR-mutated patients with advanced or metastatic NSCLC. The expected enrollment is 24 patients	NA
	Phase 2 trial (NCT00312975)	Randomized trial comparing tremelimumab with best supportive care after first-line platinum-based therapy in patients with locally advanced or metastatic NSCLC. The 87 patients enrolled received tremelimumab (n = 44) or best supportive care (n = 43)	Tremelimumab was tolerable, with safety consistent with prior studies. Among patients receiving tremelimumab, there were 4.8% partial responses and 16.6% stable diseases, compared with 0% and 14.3% patients receiving best supportive care, respectively. However, progression-free survival analysis did not demonstrate superiority of tremelimumab over best supportive care
	Phase 2 study (NCT01998126)	Trial assessing the toxicity of ipilimumab and erlotinib in EGFR-mutated patients and toxicity of ipilimumab and crizotinib in ALK-mutated patients with stage IV NSCLC. Estimated enrollment: 46 subjects	NA
	Phase 2 study (NCT00527735)	Multicenter trial including 204 chemotherapy-naïve patients with stage IIIB–IV NSCLC. Subjects were assigned to a control arm (up to six doses of placebo plus chemotherapy—paclitaxel and carboplatin), concurrent anti–CTLA-4 antibody (ipilimumab) regimen (four doses of anti–CTLA-4 antibody plus the same combination of chemotherapeutic drugs followed by two doses of placebo plus chemotherapy), or phased ipilimumab regimen (two doses of placebo plus chemotherapy followed by four doses of ipilimumab plus chemotherapy)	The authors have reported that phased ipilimumab plus chemotherapy improved progression-free survival. However, the clinical benefit was only slight
	Phase 2 study (2006-000568-95)	Randomized, noncomparative study using anti–CTLA-4 (tremelimumab) or best supportive care in patients with NSCLC (stage IIIB or IV disease) that has responded or remained stable after platinum-based therapy	NA
PD-1	Phase 3 trial (NCT01285609)	Randomized, multicenter, double-blind trial in patients with stage IV/recurrent squamous NSCLC comparing the effect of ipilimumab plus paclitaxel and carboplatin vs. placebo plus paclitaxel and carboplatin is currently recruiting patients. Estimated enrollment: 920 subjects	NA
	Phase 1 trial (NCT01295827)	Exploration of the clinical activity and efficacy of anti–PD-1 antibody (MK-3475, lambrolizumab) in subjects with locally advanced or metastatic NSCLC. The efficacy of low, medium, and high doses of the drug in combination with standard chemotherapy in participants with locally advanced or metastatic NSCLC and the effects of low and high doses of blocking antibody in treatment-naïve and previously treated participants with NSCLC with PD-L1 gene expression were also assessed	Some adverse events have been reported, but most of them were low grade. In this trial, the potential clinical benefits of the anti–PD-1 antibody are still being explored
	Phase 1 trial (NCT01840579)	Study using pembrolizumab (MK-3475) alone or in combination with cisplatin/pemetrexed or carboplatin/paclitaxel in patients with advanced NSCLC. Estimated enrollment: 30 subjects	NA

(Continued)

Table 1. (Continued)

Target	State of Clinical Development	Trial Design	Conclusions
	Phase 1 study (NCT02007070)	Open-label, nonrandomized, multicenter study of MK-3475 in subjects with PD-L1 ⁺ advanced NSCLC. Estimated enrollment: 24 subjects	NA
	Phase 1 trial (NCT01454102)	Multiaim safety study of nivolumab in combination with platinum doublet, bevacizumab maintenance, erlotinib, ipilimumab, or nivolumab alone in subjects with stage IIIB/IV NSCLC. Estimated enrollment: 412 patients	NA
	Phase 1/2 trial (NCT02039674)	Trial assessing the safety, tolerability, and efficacy of pembrolizumab (MK-3475) in combination with chemotherapy or immunotherapy in participants with locally advanced or metastatic NSCLC. Estimated enrollment: 320 subjects	NA
	Phase 2 trial (NCT01721759)	Assessment of the objective response rate in patients with advanced or metastatic squamous NSCLC treated with nivolumab (BMS-936558) after failure of two prior systemic regimens. Study ongoing (estimated enrollment: 100 patients)	NA
	Phase 2 trial (NCT01928576)	Nivolumab monotherapy after azacitidine + entinostat or after oral azacitidine (epigenetic priming study). Estimated enrollment: 120 subjects	NA
	Phase 2/3 trial (NCT01905657)	Study of low/high doses of MK-3475 (pembrolizumab) vs. docetaxel in patients with NSCLC who have experienced disease progression after platinum-based chemotherapy. The estimated enrollment is 920 subjects with PD-L1–positive tumor	NA
	Phase 3 trial (NCT00527735)	Randomized analysis of the clinical value of BMS-936558 (nivolumab) in 122 patients with NSCLC after failure of prior therapy	Objective and durable responses have been reported in patients with squamous (response rate, 33%) as well as in nonsquamous histological type (response rate, 12%)
	Phase 3 trial (NCT01642004)	Study of nivolumab (BMS-936558) compared with docetaxel in subjects with advanced or metastatic squamous NSCLC after failure of prior platinum-based chemotherapy. Estimated enrollment: 264 subjects	NA
	Phase 3 trial (NCT01673867)	Nivolumab (BMS-936558) monotherapy vs. docetaxel in metastatic nonsquamous cell NSCLC after failure of prior platinum-based chemotherapy. Estimated enrollment: 582 subjects	NA
PD-L1	Phase 1 study (NCT01846416)	Multicenter, single-arm study evaluating the efficacy and safety of MPDL3280A in advanced or metastatic PD-L1–positive NSCLC. This study is ongoing, but not recruiting participants (n = 128)	NA
	Phase 1 trial (NCT02013219)	Open-label, multicenter study assessing the safety, tolerability, and pharmacokinetics of MPDL3280A and erlotinib administered in combination to patients with NSCLC. Thirty-two patients are expected to be enrolled in this trial	NA
	Phase 1 trial (NCT00729664)	Safety, clinical activity, and tolerability assessment of anti-PD-L1 antibody (BMS-936559) in 75 patients with advanced NSCLC. Other patients with cancer were also included in this trial (55 with melanoma, 18 with colorectal cancer, 17 with renal cell cancer, 17 with ovarian cancer, 14 with pancreatic cancer, 7 with gastric cancer, and 4 with breast cancer)	Among 75 patients, 49 were included in the efficacy analysis. Five objective responses were observed at doses of 3 and 10 mg/kg, with response rates of 8 and 16%, respectively

(Continued)

Table 1. (Continued)

Target	State of Clinical Development	Trial Design	Conclusions
	Phase 1 study (NCT01375842)	Assessment of MPDL3280A, in 85 pretreated patients with advanced or metastatic NSCLC	The treatment was well tolerated and often yielded rapid, durable responses (overall response rate, 23%). Objective response was particularly pronounced in the population with the highest level of PD-L1 expression
	Phase 1 study (NCT02000947)	Open-label study evaluating the tolerability and safety of MEDI4736 in combination with tremelimumab in subjects with advanced NSCLC. Estimated enrollment: 208 patients	NA
	Phase 2 trial (NCT02031458)	Multicenter, single-arm study evaluating the efficacy and safety of MPD L3280A in patients with PD-L1 ⁺ locally advanced or metastatic NSCLC. Estimated enrollment: 635 subjects	NA
	Phase 2 trial (NCT01903993)	Multicenter, open-label, randomized trial evaluating the efficacy and safety of MPDL3280A compared with docetaxel in patients with advanced or metastatic NSCLC that failed to respond to platinum-based treatment. Enrollment: 287 subjects	NA
	Phase 3 study (NCT02008227)	Randomized trial evaluating the efficacy and safety of MPDL3280A compared with docetaxel in patients with locally advanced or metastatic NSCLC after failure with platinum-based chemotherapy treatment. Estimated enrollment: 850 patients	NA

Definition of abbreviations: ALK = anaplastic lymphoma kinase; CTLA-4 = cytotoxic T-lymphocyte-associated protein-4; EGFR = epidermal growth factor receptor; NA = not available; NSCLC = non-small cell lung cancer; PD-1 = programmed cell death protein-1; PD-L1 = programmed cell death ligand-1.

benefit from checkpoint-blocking immunotherapies (82). In patients with melanoma treated with immune checkpoint blockade agents, it has been suggested that a preexisting T cell-rich tumor microenvironment could be a predictive marker of good response to therapy (83). Similar analyses are still missing in NSCLC. PD-L1 expression on tumor cells has been reported as a potential predictive biomarker for PD-1 blockade (84), but data are still limited. Although some other associations between tumor microenvironment biomarkers and clinical activity have been reported (85, 86), they still need to be more intensively investigated to validate them as predictive markers of response or for selection of patients most likely to respond to the treatment. These new immunotherapeutic interventions represent a major breakthrough in oncology, especially for the treatment of patients with advanced or metastatic lung cancer who fail to respond to chemotherapy or who relapse. Checkpoint inhibition stands in contrast to a variety of previous trials targeting the immune system in patients with NSCLC who have largely failed in the

clinic (IFN, IL-2, vaccination, etc.) (22). One of the largest phase 3 studies in NSCLC with MAGE-A3 protein-based immunotherapy did not meet its primary end points, and a planned subanalysis using a predictive immune gene signature as a surrogate for immune contexture unfortunately could not be done. The limited success of these clinical trials could be explained by the immunosuppressive properties of the tumor microenvironment that counteract T-cell activity, but also by weak mobilization of immunity, immunoselection, or immune escape. Checkpoint blockade may, however, revive some of these failed strategies through future rational combinations to further improve clinical efficacy.

Prognostic Immune Markers in NSCLC

In a large array of various primary and metastatic tumors, it has been reported that the immune microenvironment is often associated with the clinical outcome of patients (87, 88). A worldwide task force is

currently working to establish the immunoscore as an independent prognostic marker for colorectal cancer (89).

In NSCLC, several studies have investigated the association between the presence and density of adaptive or innate immune cell populations and patient survival (Table 2). In the majority of studies, high CD3⁺, CD8⁺, or CD4⁺ T-cell infiltrations were associated with a favorable prognosis. Tregs were associated with poor survival in NSCLC. The ratio of stromal FoxP3 to CD3-positive cells was also significantly associated with a higher risk of relapse (Table 2). It is important to highlight the absence of specific markers of regulatory T cells in immunohistochemistry, and most of the investigators used FoxP3 or CD25 (also expressed at low levels by activated T cells) as surrogate markers of Tregs. As previously stated, the location of Tregs and their presence within TLSs could be of major importance in their impact on the survival of patients with NSCLC (90). One report demonstrated an inverse clinical correlation between the density of IL-17 (a cytokine produced

Table 2. Summary of Prognostic Immune Markers in Non–Small Cell Lung Cancer

Marker	Author and Year	Patients (n)	Stage(s)	Conclusions
T cells	Johnson <i>et al.</i> (2000) (91)	95	I (57%) II (18%) III (21%)	High CD3 ⁺ in tumor correlated with longer OS
	Hiraoka <i>et al.</i> (2006) (92)	109	I (61%)	Concurrent high CD4 ⁺ and CD8 ⁺ in stroma correlated with longer survival
	Kikuchi <i>et al.</i> (2007) (93)	161	II–III (39%) I (59%)	HLA class I expression correlates with longer OS in stage I
	Ruffini <i>et al.</i> (2009) (94)	1,290	II–IV (41%) I (55%) II (21%)	HLA class I expression correlated with CD8 ⁺ cells TILs (mostly CD8 ⁺ cells) in tumor correlated with better OS
	Wakabayashi <i>et al.</i> (2003) (95)	178	III A (17%) I (60%) II (13%)	High CD4 ⁺ in stroma correlated with longer OS High CD8 ⁺ in tumor correlated with shorter OS
	Al-Shibli <i>et al.</i> (2008) (96)	335	III A (27%) I (63%) II (27%)	High CD4 ⁺ in stroma correlated with longer DSS High CD8 ⁺ in stroma correlated with longer DSS
	Kawai <i>et al.</i> (2008) (97)	199	III A (10%) IV (100%)	Predominant distribution of CD8 ⁺ T cells in cancer nests as opposed to cancer stroma correlated with longer OS
	Suzuki <i>et al.</i> (2013) (98)	956	I (100%)	Densities of CD3 ⁺ , CD4 ⁺ , CD8 ⁺ , CD45RO ⁺ are not associated with RFP Relative proportion of stromal FoxP3 ⁺ to CD3 ⁺ correlated with RFP
	Goc <i>et al.</i> (2014) (40)	376	I (44%) II (27%) III (28%) IV (0.5%) ND (0.5%)	High CD8 ⁺ T cells correlated with longer survival
	Remark and Damotte, unpublished data	161	III (100%)	High CD8 ⁺ T cells correlated with longer survival
B cells	Germain <i>et al.</i> (2014) (36)	196	I (32%) II (6%) III (62%)	High density of follicular B cells correlated with longer survival
	Pelletier <i>et al.</i> (2001) (99)	113	I (58%) II (18%) III (24%)	Peritumoral CD20 ⁺ correlated with longer survival
	Al-Shibli <i>et al.</i> (2008) (96)	335	I (63%) II (27%) III A (10%)	High CD20 ⁺ in stroma correlated with longer DSS
Th17 cells	Suzuki <i>et al.</i> (2013) (98)	956	I (100%)	Densities of CD20 ⁺ are not associated with RFP
	Chen <i>et al.</i> (2010) (100)	52	I–II (63%) III (37%)	High IL-17 ⁺ cell densities correlated with poor survival
Tregs	Suzuki <i>et al.</i> (2013) (98)	956	I (100%)	Densities of FoxP3 ⁺ cells are not associated with RFP Relative proportion of stromal FoxP3 ⁺ to CD3 ⁺ correlated with RFP
	Tao <i>et al.</i> (2012) (101)	87	ND	High Treg densities correlated with poor OS and DFS
	Shimizu <i>et al.</i> (2010) (102)	100	I (68%) II (14%) III (18%)	High FoxP3 ⁺ correlated with shorter time to recurrence COX-2 expression correlated with shorter time to recurrence
	Petersen <i>et al.</i> (2006) (103)	64	I (100%)	COX-2 expression correlated with FoxP3 ⁺ infiltration High proportion of FoxP3 ⁺ among TILs in tumor correlated with shorter DFS
NK cells	Platonova <i>et al.</i> (2011) (104)	86	I (83%) II (10%) IV (7%)	Presence of NKp46 ⁺ NK cells did not impact the clinical outcome
	Johnson <i>et al.</i> (2000) (91)	95	I (57%) II (18%) III (21%)	High CD57 ⁺ NK cell density tended to correlate with longer OS (<i>P</i> = 0.07)
	Takanami <i>et al.</i> (2001) (105)	150	I (55%) II (14%) III (31%)	High CD57 ⁺ NK cells correlated with longer OS in patients with adenocarcinoma

(Continued)

Table 2. (Continued)

Marker	Author and Year	Patients (n)	Stage(s)	Conclusions
DCs	Al-Shibli <i>et al.</i> (2009) (106)	335	I (63%) II (27%) III (10%)	High stromal CD56 ⁺ cells correlated with improved DSS
	Villegas <i>et al.</i> (2002) (107)	50	I (82%) II (12%) III (6%)	CD57 ⁺ cells correlated with longer survival in patients with squamous cell carcinoma
	Dieu-Nosjean <i>et al.</i> (2008) (18)	74	I (84%) II (16%)	High DC-LAMP ⁺ mature DCs correlated with longer survival
	Inoshima <i>et al.</i> (2002) (108)	132	I (51%) II (17%) III (25%) IV (7%)	High S-100 ⁺ DCs correlated with longer survival
	Goc <i>et al.</i> (2014) (40)	376	I (44%) II (27%) III (28%) IV (0.5%) ND (0.5%)	High DC-LAMP ⁺ mature DCs correlated with prolonged survival
	Zeid <i>et al.</i> (1993) (109)	130	ND	High S-100 ⁺ Langerhans cells (LCs) in tumor were associated with enhanced survival
	Germain <i>et al.</i> (2014) (36)	196	I (32%) II (6%) III (62%)	DC-LAMP ⁺ mature DCs correlated with longer survival
	Johnson <i>et al.</i> (2000) (91)	95	I (57%) II (18%) III (21%)	High S-100 ⁺ LCs in tumor correlated with longer OS
	Sautés-Fridman <i>et al.</i> (2011) (110)	74	I (84%) II (16%)	High CD1a ⁺ LCs and CD14 ⁺ CD68 ^{low} interstitial DCs were associated with longer DSS
	Al-Shibli <i>et al.</i> (2009) (106)	335	I (63%) II (27%) III (10%)	High stromal CD1a ⁺ DCs correlated with improved DSS
TAMs	Remark and Damotte, unpublished data	161	III (100%)	High DC-LAMP ⁺ mature DCs correlated with longer survival
	Chen <i>et al.</i> (2003) (111)	35	I (40%) II (11%) III (49%)	TAMs in stroma correlated with shorter OS
	Kawai <i>et al.</i> (2008) (97)	199	IV (100%)	Predominant infiltration of TAMs in cancer nests was a significant predictor of poor survival
	Zeni <i>et al.</i> (2007) (112)	47	I (51%) II-IV (49%)	IL-10-high TAMs associated with shorter OS
	Ohri <i>et al.</i> (2009) (113)	40	I (65%) II (20%) III (15%)	High number of M1 macrophages correlated with longer survival
	Ho <i>et al.</i> (2008) (114)	68	I (35%) II (22%) III (43%)	TREM-1 expression in macrophages correlated with shorter DFS and OS
	Takanami <i>et al.</i> (1999) (115)		I (50%) II (4%) III (36%) IV (10%)	High number of CD68 ⁺ TAMs correlated with longer OS
	Al-Shibli <i>et al.</i> (2009) (106)	335	I (63%) II (27%) III (10%)	High CD68 ⁺ were not associated with DSS
	Welsh <i>et al.</i> (2005) (116)	162	I (49%) II (27%) IIIa (22%) IIIb/IV (2%)	High stromal and tumor islet CD68 ⁺ TAMs correlated with OS
	Kim <i>et al.</i> (2008) (117)	144	I (55%) II (17%) III (26%) IV (2%)	TAMs in tumor correlated with prolonged OS
Ohtaki <i>et al.</i> (2010) (118)	170	IA (56%) IB-IIIa (44%)	Significant association between high numbers of CD204 ⁺ macrophages and poor outcome	
Ma <i>et al.</i> (2010) (119)	100	I (35%) II (20%) III (35%) IV (10%)	High CD68 ⁺ HLA-DR ⁺ M1 macrophages were associated with good outcome. CD68 ⁺ CD163 ⁺ M2 macrophages had no prognostic value	

(Continued)

Table 2. (Continued)

Marker	Author and Year	Patients (n)	Stage(s)	Conclusions
Neutrophils	Carus <i>et al.</i> (2013) (120)	335	I (65%) II (20%) III (15%)	CD163 ⁺ macrophage density was not correlated with survival
	Ilie <i>et al.</i> (2012) (121)	632	I (44%) II (29%) III (27%)	High CD66 ⁺ neutrophil densities were associated with higher risk of relapse
	Carus <i>et al.</i> (2013) (120)	335	I (65%) II (20%) III (15%)	CD66b ⁺ neutrophil count was not correlated with survival

Definition of abbreviations: COX-2 = cyclooxygenase-2; DC-LAMP = dendritic cell lysosome-associated membrane protein; DFS = disease-free survival; DSS = disease-specific survival; ND = not determined; NK = natural killer; OS = overall survival; RFP = relapse-free period; TAMs = tumor-associated macrophages; TILs = tumor-infiltrating lymphocytes; Treg = regulatory T cells.

mainly by Th17 cells)—positive cells and patient survival (Table 2). This finding is in accordance with the proinflammatory properties of Th17 cells. In contrast to T cells, the clinical impact of B cells is poorly understood. Every stage of B-cell differentiation has been observed in NSCLC. In general, high densities of B cells were correlated with increased survival (Table 2). We can hypothesize that B cells play a role in antitumor immunity, perhaps by capturing and presenting tumor antigens to T cells directly or by generating tumor antigen-specific antibodies that target tumor antigens in the form of immune complexes to professional APCs. As previously stated, NK cells are also present in the microenvironment of NSCLC. As nonspecific markers have been used in the majority of studies, the clinical impact of NK cells is poorly understood. However, using the Nkp46 marker, it has been suggested that the density of NK cells does not correlate with clinical outcome in early-stage NSCLC (Table 2). The altered phenotype of NK cells in the tumor microenvironment could explain this absence of clinical impact. As reported for other solid cancers (11), tumor-associated macrophages (TAMs) represent another major component of the immune microenvironment of NSCLC. In an overly simplistic manner, TAMs have been classified as “proinflammatory” M1 macrophages with antitumor activity, and “proangiogenic and immunosuppressive” M2 macrophages with protumor activity. In NSCLC, TAMs with a M1 phenotype were associated with good survival and high M2 macrophage number correlated with a poor prognosis (Table 2). However, a more comprehensive analysis of the

clinical impact of TAMs is still needed. Neutrophils represent a significant portion of infiltrating inflammatory cells, but little is known about their role in NSCLC. It has been suggested that high neutrophil counts are associated with a higher risk of relapse but not with survival (Table 2). Their potentially different, specialized functional phenotype has never been taken into account in these analyses. DCs are major regulators of immune response and can elicit T-cell responses unlike any other APCs. In NSCLC, mature DCs are located exclusively within TLSs and are associated with a good prognosis (Table 2). Because the density of mature DCs and the number of TLSs correlate with each other, it has been proposed that the presence of TLSs is associated with a good prognosis in patients with NSCLC (18).

Some studies have highlighted that the positive impact of T cells, DCs, or B cells might be limited to more advanced stages of disease (stages II–IV). This interesting observation could be linked to the good efficacy of curative surgery at early stages and the potential beneficial effect of the immune response in inducing systemic protection when hematogenous dissemination occurs at later stages.

In conclusion, the immune infiltrates are of major importance in the clinical outcome of patients with NSCLC (Table 2), and the immune contexture should be taken into account in the clinical management of patients with cancer.

Conclusions

During lung cancer progression, tumor cells interact with their microenvironment,

where local cancer antigen-specific immune responses shape their eventual destiny. There is now increasing evidence that the NSCLC immune contexture, defined as the nature, location, density, organization, and functional orientation of a natural *in situ* immune reaction, represents a major player in the development and progression of malignant disease. Some elements of this immune ecosystem and particularly the presence of lymphoid-like structures correlate with survival. Tumors in compensation develop escape mechanisms to avoid destruction. This offers potential targets for therapies. Blocking antibodies targeting the checkpoint inhibitors have shown promising results in several solid tumors. Their efficacy is being tested actively in several clinical trials in NSCLC. We are also actively pursuing strategies to alter the microenvironment of NSCLC by injection of Toll-like receptor agonists at the tumor site, followed by evaluation of immune contexture from surgical specimens. We believe that evaluating the immune contexture represents an important tool to identify subsets of patients with high risk of relapse, and to predict outcome in the context of treatment. Standardized immunopathological assessment in the form of a prognostic and/or predictive score should now be validated in large consortium-driven studies and implemented to help clinicians to decide which therapeutic strategy to choose. ■

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