The Role of Tumor-Infiltrating Lymphocytes in Development, Progression, and Prognosis of Non–Small Cell Lung Cancer

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ABSTRACT

A malignant tumor is not merely an accumulation of neoplastic cells, but constitutes a microenvironment containing endothelial cells, fibroblasts, structural components, and infiltrating immune cells that impact tumor development, invasion, metastasis, and outcome. Hence, the evolution of cancers reflects intricate cellular and molecular interactions between tumor cells and constituents of the tumor microenvironment. Recent studies have shed new light on this complex interaction between tumor and host immune cells and the resulting immune response. The composition of the immune microenvironment differs across patients as well as in cancers of the same type, including various populations of T cells, B cells, dendritic cells, natural killer cells, myeloid-derived suppressor cells, neutrophils, and macrophages. The type, density, location, and organization of immune cells within solid tumors define the immune contexture, which has proved to be a major determinant of tumor characteristics and patient outcome. Lung cancer consists mostly of non–small cell lung cancer (85%); it is our most deadly malignant disease, with the 5-year survival rate being merely 15%. This review focuses on the immune contexture; the tumor-suppressing roles of tumor-infiltrating lymphocytes; and the relevance of this immune contexture for cancer diagnostics, prognostication, and treatment allocation, with an emphasis on non–small cell lung cancer.

Keywords: Tumor microenvironment; TILs; Prognosis; Immune context; Immunoscore; NSCLC

Introduction

Until recently, the principal focus in cancer research was the malignant cell, with relative neglect of the tumor's microenvironment represented by endothelial, stromal, and immune cells; components of the extracellular matrix; and an abundance of mediators.[1] Already 50 years ago, while still discussing whether the immune system had positive, negative, or no effects on tumor development, Burnet and Thomas proposed the hypothesis of immunological surveillance, in which the immune system acts as a sentinel by detecting and eliminating nascent transformed cells and thereby protecting against cancer development.[2,3] This hypothesis

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was, however, abandoned shortly afterwards owing to the absence of strong experimental evidence.

During the past 15 years, the groundbreaking adaptive immunity studies in mice by Schreiber et al. have clearly proved the existence of cancer immunosurveillance.[4–7] This concept has been further refined into cancer immunoediting, incorporating a broader view of tumor–immune system interaction.[8] During this progress, we have witnessed a radical change of view on malignancy, from it being an autonomous cellular disease comprising six biological capabilities[9] to it being a regulated disease involving the immune components of its microenvironment.[10] Through interactions with tumor cells, immune cells will induce tumor fates according to the three E phases (elimination, equilibrium, or escape) by activation of innate and adaptive immune responses.[6] The work by Schreiber et al. definitely altered the field of cancer immunology, and "evading immune destruction" was added as an emerging hallmark in the revision of "The Hallmarks of Cancer."[10]

Early clinical studies revealed that tumor-infiltrating lymphocytes (TILs) had a major impact on the clinical course of several cancers.[11–18] More recently, Fridman et al. reviewed the effect of T cells on the clinical outcome of a variety of solid cancers and found that a strong infiltration of TILs was associated with a positive clinical outcome in several cancers, including melanoma as well as head and neck, breast, bladder, urothelial, ovarian, colorectal, renal, prostatic, and lung cancer.[19] More specifically, the most consistent positive prognostic impacts were demonstrated for T cells, especially cytotoxic T cells, memory T cells, and T helper cells 1.[19] These data not only elucidate the relationship between immunity and cancer but will also have consequences for the management of malignant disease. In colorectal and lung cancer, it has more recently been established that TILs can differentiate prognosis within each tumor, node, and metastasis (TNM) stage,[20,21] rendering type and density of TILs to be powerful prognostic factors complementing or even outperforming pathological criteria alone.

In 2011, Goldstraw et al. declared that the TNM classification had stood the test of time and remained the most powerful prognostic instrument for lung cancer.[22] In line with the rapidly increasing documentation of the profound prognostic impact by TILs in malignant tumors, including non–small cell lung cancer (NSCLC), one may argue that this perception needs to be revised.

Cancer Immunosurveillance, Immunoediting, and Immune Contexture

The immune infiltrate, in lung cancers as well as in other malignancies, has been shown to comprise adaptive and innate immune cells.[19,23,24] Besides, immune infiltrates are heterogeneous across both tumor types and patients with cancer.[19] All immune cell types may be present in a tumor, and they include macrophages, neutrophil granulocytes, dendritic cells, mast cells, natural killer (NK) cells, naive and memory lymphocytes, B cells, and effector T cells (T helper cells 1, 2, and 17; regulatory T [Treg] cells; T follicular helper cells; and cytotoxic T cells). These may be localized in the tumor core, invasive margin, or adjacent tumor stroma. The immune cell type, density, and location, as well as the functional orientation of involved immune cell populations constitute the *immune contexture*, whereas chemokines and cytokines are involved in shaping it.[25]

Observational studies linked to clinical outcome data have suggested both the existence and relevance of the immune surveillance phenomenon in humans, which was characterized earlier by Schreiber et al.[7] Through this dynamic process, the immune system not only protects against cancer development but also appears to shape the character of emerging tumors according to the concept of immunoediting.[26] Herein, we will convey how the immune system plays a dual role in cancer progression as it can not only suppress tumor growth by eliminating cancer cells but also promote tumor growth by selecting cancer cells that can evade surveillance.

The three phases of immunoediting are elimination, equilibrium, and escape (Fig. 1).[6,8,27] In the *elimination* phase, innate and adaptive cells of a competent immune system can together detect and destroy early tumors before they become clinically apparent. Such tumor cells are immunogenic, expressing antigens that differentiate them from nontransformed cells, and the balance is toward antitumor immunity. Cancer cells that survive the elimination phase enter the *equilibrium* phase, in which the immune system holds the tumor in a state of dormancy. Some tumor cells undergo genetic/epigenetic changes, and owing to constant immune pressure, new evolving tumor cells can resist immune recognition. In this phase there is a balance between antitumor and tumor-promoting cytokines. During the *escape* phase, tumor cells can induce an immunosuppressive state through production of cytokines and growth factors, as well as by recruiting immunosuppressive cells, leading to an impairment of effector T cells. Next, tumor cells evade immune recognition and the immune system fails to restrict tumor growth, eventually causing clinical disease. These tumor cells generally express molecules with increased resistance, survival, immunosuppression, and angiogenesis, which again induce the generation of immunosuppressive cells (e.g., Treg cells and myeloid-derived suppressor cells) and cytokines. Progression during the escape phase explains the paradoxical observation of tumor development in immunocompetent

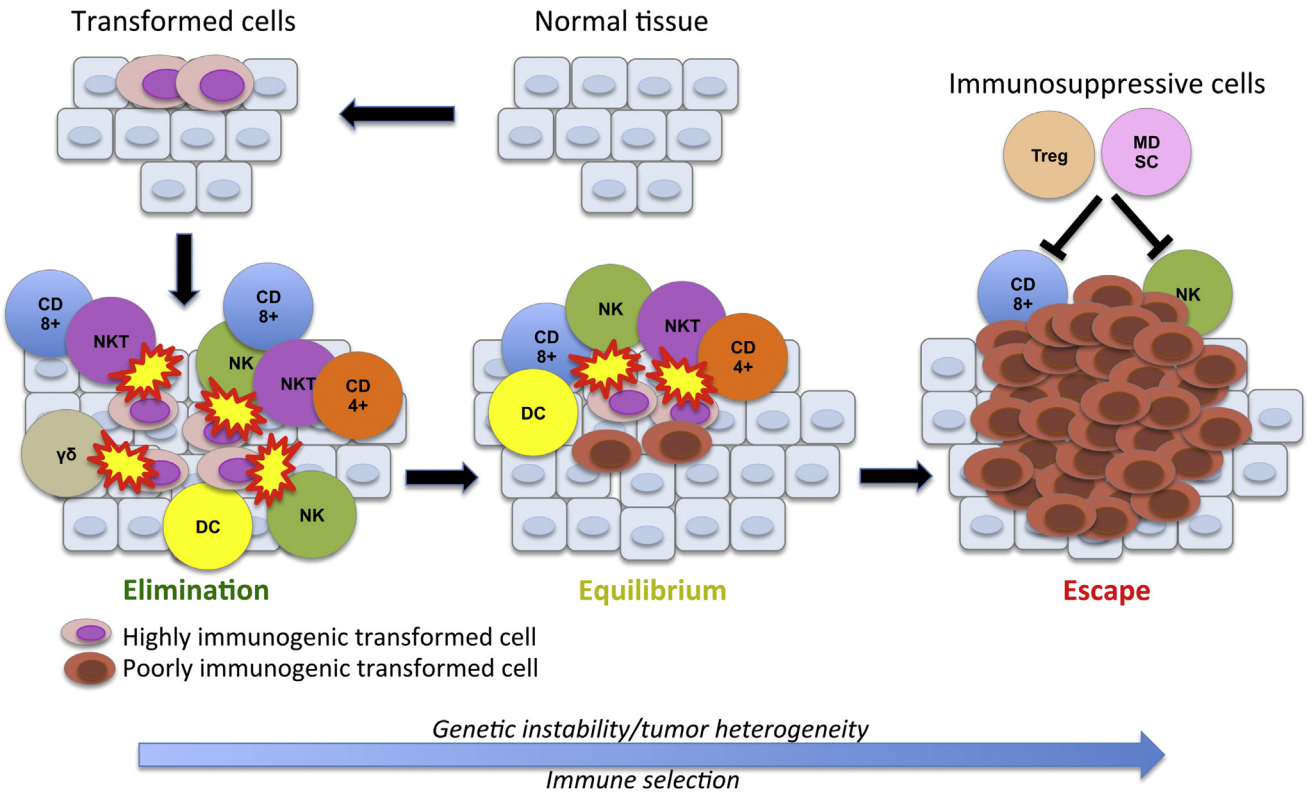

Figure 1. Immunosurveillance and immunoediting. There is an integration of immunoediting and oncogenesis during cancer progression. Oncogenesis leads to transformed cells, which are attacked by immune cells owing to neoantigen presentation. During immunosurveillance, cancer immunoediting incorporates three phases: elimination, equilibrium, and escape. In the *elimination* phase the cancer immunosurveillance successfully eradicates cancer cells of the developing tumor. During the *equilibrium* phase the tumor cells that have survived the immunosurveillance and the host immune system are in balance. In the *escape* phase the tumor cells have evaded detection and elimination by the host immune system. The immunosurveillance imposes a selection of transformed cells that acquire tactics to escape control. Their genetic instability facilitates evolution of strategies for immune evasion or suppression, which may tilt the tumor microenvironment from hostile to supportive for the transformed cells. At some point, a state of equilibrium may be achieved, corresponding to a clinically occult dormant disease. Further iteration of evasion mechanisms may ultimately drive immune suppression beyond the local microenvironment, accomplishing immune escape and in this manner licensing invasive and metastatic behavior. $CD8^+$, cytotoxic T cells; $CD4^+$, T helper cells; NK, natural killer cells; NKT, natural killer T cells; DC, mature dendritic cells; Treg, regulatory T cells; MDSC, myeloid-derived suppressor cells; $\gamma\delta$, gamma delta T cells. Adapted from Mittal et al.,[8] Dunn et al.,[26] and Prendergast.[28] Adapted with permission from Sakakura and Chikamatsu.[29]

individuals.[6] For updated and detailed insights into cancer immunoediting, see the review by Mittal et al.[8]

The immunoediting process describes the evolution of interaction between immune and tumor cells during cancer development, and it can be applied to NSCLC as well as to other tumor types. It is documented that lung cancers can be immunogenic tumors, eliciting different serum antibody responses, and as reported for other solid tumors, the immune microenvironment of NSCLC can have a strong prognostic value.[19] Besides, data support the concept of an immune system capable of recognizing and eliminating malignant cells.[19]

The emergence of cancer immunoediting as a framework to understand the extent of the immune system's interaction with cancer has led to an escalating interest in this field, with at least a fivefold increase in published papers within the research area since 2004.[30]

Immunosurveillance and Tertiary Lymphoid Structures

For most malignant cell types and studies, $CD8^+$ cytotoxic T cells and T helper cells 1 have an overall positive prognostic effect, whereas Treg cells have a detrimental prognostic effect.[19] The mechanisms behind the positive correlation between TILs and the prognosis of patients with NSCLC have been controversial. Lately, accumulating evidence has indicated that adaptive immune responses can be initiated in the tumor environment independent of secondary lymphoid organs (e.g., lymph nodes).[31–33]

Whereas immune cells in general are randomly distributed within the tumor, in 2008 Dieu-Nosjean et al. published evidence supporting the formation of ectopic lymph node–like specified structures, so-called tertiary

lymphoid structures (TLSs) in NSCLC tumors (Fig. 2).[19,34,35] These resemble and function like secondary lymphoids, and antigen presentation take place in them.[36,37] TLSs harbor all the characteristics of active immunological sites, with T- and B-cell zones and dendritic cells (DCs), and they are surrounded by specialized blood vessels termed *high endothelial venules* (HEVs), which make contact with lymphocytes and a germinal center.[38] The T-cell area comprises clusters of T cells and mature DCs (mDCs). The immunological functions of TLSs in cancer are just beginning to be unraveled. In humans, these structures are not present in adults under normal conditions, with the exception of mucosa-associated lymphoid tissue in the gut.

One may ask: What is the potential immunologic contribution of TLSs in a microenvironment such as malignant tissues that is generally considered immunosuppressive? Goc et al found that NSCLC-associated TLSs present a specific chemoattractant signature associated with T-cell infiltration.[39] They also observed that HEVs colocalized with TLSs, indicating that these vascular structures provide a key hub for the immigration of peripheral blood immune cells into the tumor tissue. In a clinical study of 458 patients with stage I–III NSCLC, the same research group assessed the impact of TLSs on the characteristics of intratumoral immune contexture and clinical outcome.[40] They found that a high density of mDCs correlated with a high infiltration of TILs,

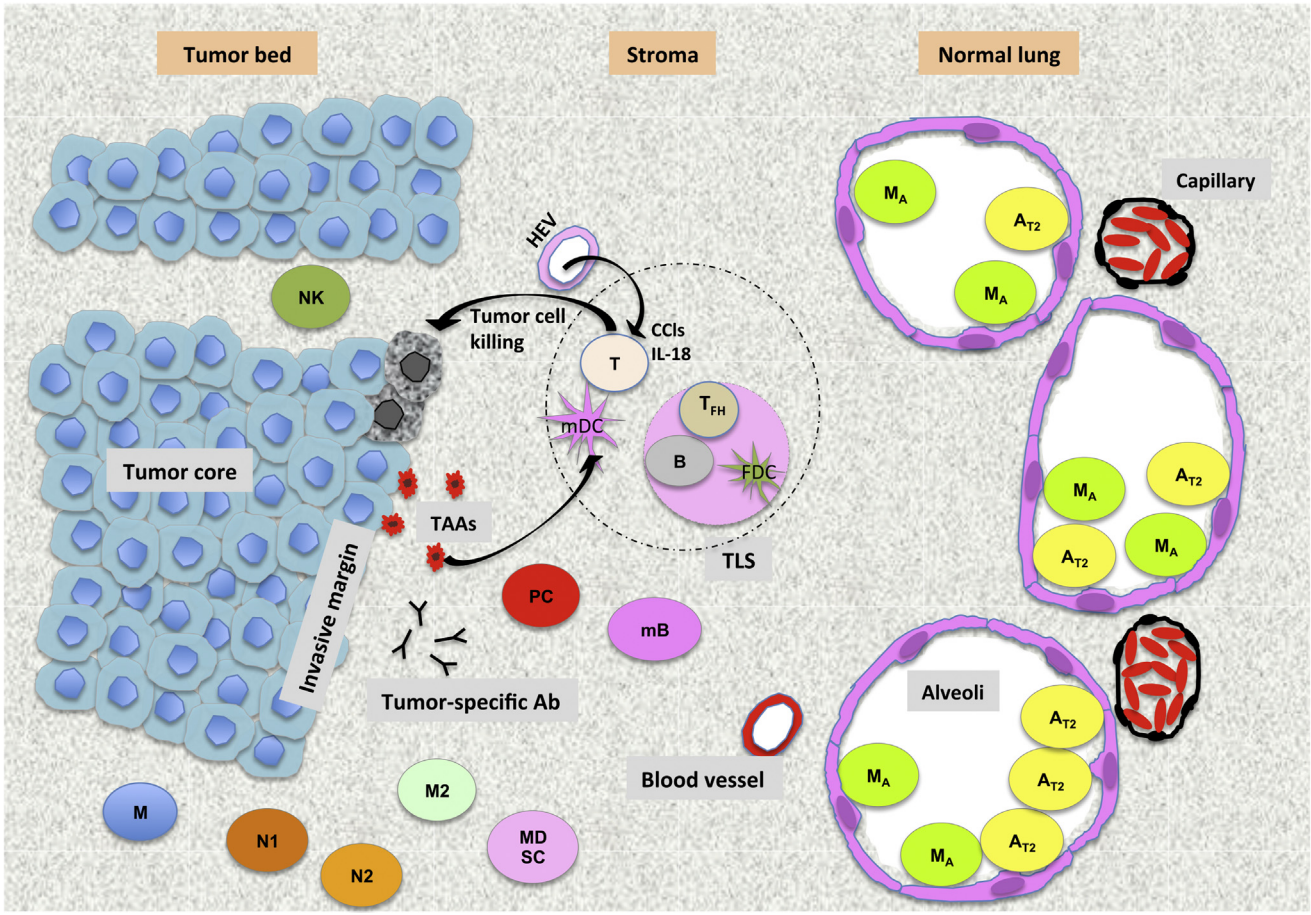

Figure 2. The immune contexture in non-small cell lung cancer includes the tumor core, invasive margin, tertiary lymphoid structures (TLSs), and tumor microenvironment, as well as the distribution of various immune cells. The immune microenvironment in lung tumors comprises T cells (T), B cells (B), natural killer cells (NK), mature (mDCs) and immature dendritic cells (follicular dendritic cells [FDCs]), tumor-associated macrophages [TAMs, type 2 (M2)], myeloid-derived suppressor cells (MDSCs), neutrophils (N1, antitumorigenic; N2, protumorigenic), and mast cells (M). The great majority of immune cells are found at the interface between the tumor and the normal tissue (invasive margin), and some are organized into tertiary TLSs. The latter are considered a gateway for the entrance of immune cells from the blood to the tumor (by means of 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; TAAs, tumor-associated antigens; mB, memory B cell; PC, plasma cells; $T_{FH,}$ follicular helper T cell; A_{T2}, alveolar type II cells; M_A, alveolar macrophage. Adapted from Fridman et al.[19] Adapted with permission from Remark et al.[34]

primarily of the effector memory type of $CD8^+$ T cells. mDC density also correlated with a specific molecular immune signature characterized by an overexpression and coregulation of genes influencing T-cell activation, polarization of T helper cells 1, and cytotoxic orientation. In general, these results contradict the current view of the established cancer microenvironment as solely an immunosuppressive milieu and indicate that TLSs orchestrate a specific and coordinated immune contexture in NSCLC.

It has been hypothesized that TLSs may potentiate the antitumor response by maintaining a chronic restimulation of infiltrating $CD8^+$ T cells, thus promoting a humoral immune response.[41] In all cases, the density of TLSs correlates with a favorable prognosis, as does the presence of associated HEVs, mDCs, and T helper 1 cells.[33,42–44] TLSs modulate the clinical impact of $CD8^+$ T cells, leading to a significantly improved survival in cases of NSCLC exhibiting high densities of both intratumoral $CD8^+$ T cells and TLS-associated mDCs versus high intratumoral $CD8^+$ T cells and low mDC density.[45] Besides being observed in NSCLC, TLSs have also been observed in several other cancers, such as colorectal, breast, ovarian, renal cell, and pancreatic cancer.[45]

Prognostic Immune Markers in NSCLC

Immune cells in lung cancer microenvironments primarily comprise T cells, macrophages, and mast cells, whereas plasma cells, NK cells, and myeloid-derived suppressor cells are relatively rare.[1,46–48] Although lymphocytes constitute two-thirds of immune cells, 80% of these are T cells expressing various activation antigens.[47] As in the case of M1 and M2 macrophages, neutrophils have also been found to exist in both antitumorigenic (N1) and protumorigenic forms (N2).[49,50]

At the beginning of the millennium there were some limited translational NSCLC studies on the prognostic role of immune cells, with somewhat contradictory results.[34,51–61] In 2011, Gooden et al. presented a systematic review with a meta-analysis of the prognostic influence of TILs in cancer, including in NSCLC.[62] They reported that $CD3^+$ and $CD8^+$ TILs correlated with improved overall survival (hazard ratios of 0.58 and 0.71, respectively). $FOXP3^+$ Treg cells had no significant effect on survival (hazard ratio 1.19) but tended toward a worse prognosis. More recently, Fridman et al. reviewed 124 translational cancer studies, including studies of NSCLC, and concluded that a strong infiltration of TILs was generally associated with a superior outcome.[19] The most consistent positive prognostic impacts were associated with cytotoxic T cells ($CD8^+$), memory T cells ($CD45RO^+$), and T helper cells 1.[19] Judging by these data, TILs appeared to be important players for in situ immunity of cancers.

On the other hand, it should be noted that lung cancers may progress despite the presence of TILs, as effector immune cells may be anergic with reduced functions in the lung cancer microenvironment,[24,63–65] or the 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.[66] In addition, it has been observed that lung cancer cells are able to produce immunosuppressive factors in the tumor microenvironment (soluble forms of major histocompatibility class I chain-related molecule A, cytokines, and chemokines), impairing $CD8^+$ T-cell–mediated tumor cell lysis or promoting tumor cell progression or survival.[67–73]

NSCLC and TILs

In the literature, there are 17 larger studies (sample size $N > 200$) investigating the prognostic impact of TILS in NSCLC.[14,18,40,74–87] Sixteen and 13 of these studies have been published since 2008 and 2011, respectively, clearly demonstrating the rapidly increasing interest within this topic. For details on study cohort size, included cell type, pathological TNM stage (pStage), and assessed tissue compartment and immune cells (markers), see Table 1. $CD8^+$ TILs were examined in 14 of 17 studies, whereas $CD3^+$ and $CD4^+$ TILs where assessed in six and five studies, respectively. In two of the included studies, TILs were not specified because assessments were based on hematoxylin and eosin staining alone. Three of the studies included validation cohorts.[77,84,86]

As $CD8^+$ T cells also constitute most of the $CD3^+$ category, these cells have consistently been associated with a beneficial prognostic impact in all studies based on CD markers (see Table 1). In half of the studies, $CD8^+$ T cells in fact mediated an independent positive prognostic impact. It should be noted that in the study by Ruffini et al., tumor specimens were stained for $CD3^+$, $CD8^+$, $CD4^+$, and $CD20^-$ but the analyses were performed for all TILs and not according to T cell type.[83] In the study by Donnem et al., one training set and three validation cohorts with a total of 797 patients were examined.[77] In that study, density of $CD8^+$ T cells was assessed in stroma only and showed a strong independent positive prognostic impact on disease-free survival, disease-specific survival, and overall survival. The method used for assessing the $CD8^+$ cell density in stromal compartments is briefly presented in Figure 3. Rapidly accumulating research data strongly document that high levels of tumor-infiltrating cytotoxic $CD8^+$ and $CD3^+$ T cells assessed in the stromal or tumor epithelial compartment of NSCLC are associated with significant beneficial patient outcomes mediated by immunity toward the tumor.[88] Furthermore, stromal assessments of TILs appear to have a superior prognostic impact when compared with

Table 1. Prognostic Impact of Immune Cells in Larger (N > 200) NSCLC Studies

Study	N	Histologic Diagnosis	P Stage	Markers	Tissue Compartment	TMA vs. WS	Prognostic Impact (PI) by TILs?	PI within pStage?
Johnson et al. (2000)[14]	710	NOS	I-III	CD3, CD8, CD57, CD68	Intraepithelial and stromal	WS	Total cohort (HE), TILs no significant PI. IHC analysis was performed (N = 95). Epithelial CD3 pos PI for OS (univariate)	No
Al-Shibli et al. (2008)[18]	335	ADC, SCC, NOS	I-IIIA	CD4, CD8, CD20	Intraepithelial and stromal	TMA	Stromal and epithelial CD4 and CD8 correlate with improved DSS. Both are independent PIs in stroma	No
Ruffini et al. (2009)[83]	1290	ADC, SCC, NET, SCLC	I-IIIA	TILs (CD3, CD4, CD8, CD20)	Intraepithelial	WS	PI assessed for total TILs. Independent PI overall and for SCC	Partly
Al-Shibli et al. (2010)[74]	335	ADC, SCC, NOS	I-IIIA	CD3, CD117, CD138	Intraepithelial and stromal	TMA	Stromal + epithelial CD3 correlates with improved DSS. Stromal CD3 has independent pos PI	Partly
Kilic et al. (2011)[81]	219	ADC, SCC, NOS	I	TILs (NOS)	Intraepithelial and stromal	WS	TILs (total) correlate with reduced recurrence rate and prolonged DFS (univariate)	Only stage I
Horne et al. (2011)[78]	273	ADC, SCC, NOS	IA	TILs (NOS)	Intraepithelial and stromal	WS	Total TILs show pos PI (RFS, univariate)	Only stage I
Sterlacci et al. (2012)[85]	383	ADC, SCC, NOS	I-IV	CD4, CD8	Intraepithelial	TMA	Low CD4/CD8 ratio pos PI for ADC in stage I (univariate)	Partly
Ilie et al. (2012)[79]	632	ADC, SCC, NOS	I-III	CD8, CD66b	Intraepithelial (central tumor)	TMA	CD8 not pos PI. Low CD66b/CD8 ratio has pos independent PI	No
Kayser et al. (2012)[80]	232	ADC, SCC, NOS	I-IV	CD3, CD8, CD4/CD25	Intraepithelial and stromal	TMA	High stromal CD3 and CD4/CD25 ratio favor better prognosis (univariate). High stromal CD4/CD25 ratio has independent PI	No
Suzuki et al. (2013)[86]	956	ADC	I	FOXP3, CD3, CD4, CD8, CD45RO	Intraepithelial and stromal	TMA	High FOXP3/CD3 ratio has negative PI. FOXP3-mediated protumor environment, but is overcome by high stromal CD3	Only stage I
Goc et al. (2014)[41]	458	ADC, SCC, NOS	I-IV	CD8, mDC	Intraepithelial and stromal	WS	In TLS, mDC density correlates with CD8 activation, Th phenotype, and cytotoxic orientation	No
Alifano et al. (2014)[75]	303	ADC, SCC, NOS	I-IV	CD8, CD1A	Intraepithelial and stromal	WS	High CD8 cell count shows reduced RR = 0.37. CD8 T-cell density has independent pos PI	Partly
Schalper et al. (2015)[84]	552	ADC, SCC, NOS	I-IV	CD3, CD8, CD20	Intraepithelial, stromal, total	TMA	Total CD8 has independent pos PI in both cohorts. Total CD3 has independent pos PI in one cohort	No
Kim et al. (2015)[82]	331	SCC	I-III	CD8, PD-1, PD-L1	Intraepithelial	TMA	Cases with high PD-L1 had also high CD8 cell infiltration. Cases with high CD8 and PD-1 TILs had longer DFS	No
Donnem et al. (2015)[20]	797	ADC, SCC, NOS	I-IIIA	CD8	Stromal	TMA	CD8 and pStage has independent. pos PI in whole cohort, all end points. CD8 has significant PI within each pStage (univariate)	Yes
Koh et al. (2015)[87]	497	ADC	I-III	CD8	Intraepithelial and stromal	TMA	CD8 TILs have pos PI. High CD8 count is significantly associated with increased DFS	No
Djenidi et al. (2015)[76]	101	ADC, SCC, NOS	I	CD3, CD8, CD103	Intraepithelial and stromal	WS	CD103 TILs have pos PI, promoting CTL. CD8CD103 TILs lead to activation-induced cell death.	No

ADC, adenocarcinoma; CD, cluster of differentiation; CTL, cytotoxic lymphocyte; DFS, disease-free survival; DSS, disease-specific survival; HE, hematoxylin and eosin staining; IHC, immunohistochemical; mDC, mature dendritic cells; NET, neuroendocrine tumors; NOS, not otherwise specified; NSCLC, non-small cell lung cancer; OS, overall survival; PI, prognostic impact; PD-1, programmed death receptor 1; PD-L1, programmed death ligand 1; pos, positive; pStage, pathological stage; RFS, relapse-free survival; RR, Relative risk; SCC, squamous cell carcinoma; SCLC, small cell lung cancer; Th, TIL helper cell; TILs, tumor-infiltrating lymphocytes; TLS, tertiary lymphoid structures; TMA, tissue micro array; WS, whole slide.

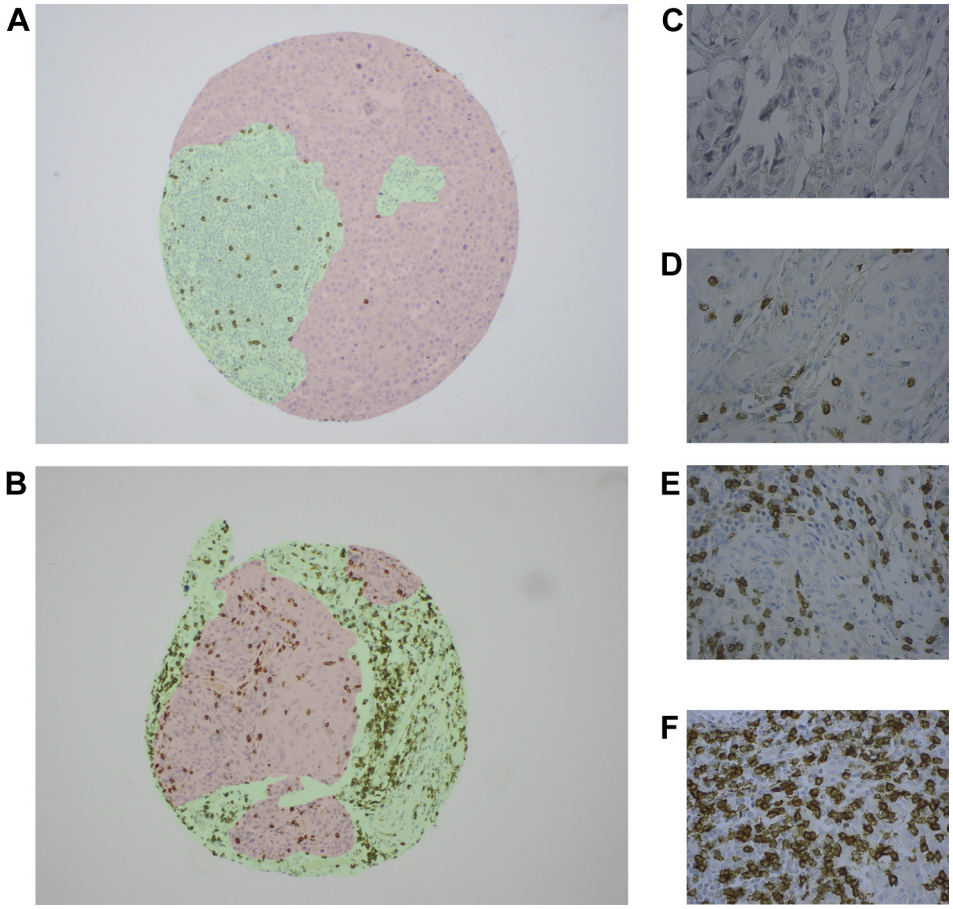

Figure 3. Representative examples of CD8 immunostaining in non-small cell lung cancer tissue microarray (TMA) cores. (*A* and *B*) In the large majority of non-small cell lung cancer tissues, histologic examination will show a mixture of epithelial tumor tissue (*red*) and tumor-related stroma (*green*). (*C*–*F*): Within each TMA core, the percentages of $CD8^+$ lymphocytes (*stained brown*) in comparison with the total amount of nucleated cells in the stromal compartments (*green*) can be estimated. In our experience with TMAs, the determination of $CD8^+$ appears to be most reliable when assessed in the stromal (*green*) compartment. The magnified panels constitute examples of a negative $CD8^+$ score (*C*), low-density score ($\leq$25% $CD8^+$ cells [*D*]), intermediate-density score (>25% to $\leq$50% $CD8^+$ cells [*E*]), and high-density score (>50% $CD8^+$ cells [*F*]) in tumor stroma at $\times$400 magnification.

epithelial assessments, which has recently been supported by a meta-analysis of 29 small and large studies of the prognostic role of various TILs in lung cancer.[88]

Development of Immunoscore and TNM-Immune in Cancer

Since the host immune system appeared to mediate a significant prognostic role in cancer, Galon et al. have led the translational research development within this field in colorectal cancer.[21,89–91] They have developed a remarkable diagnostic tool that uses assessments of $CD8^+$ and $CD3^+$ TILs in colorectal cancer tissue to create an "Immunoscore" as an essential prognostic and predictive supplement to TNM classification; it is designated TNM-Immune (TNM-I). Their findings suggest that the Immunoscore classification provides prognostic significance superior to that of the Dutch Association of Comprehensive Cancer Centers/Union for International Cancer Control TNM classification system, as a multivariate Cox analysis revealed the immune criteria to remain highly significant whereas the TNM variables and tumor differentiation did not.[21] This publication led to an editorial in *Journal of Clinical Oncology* with the title "TNM Staging in Colorectal Cancer: T is for T cell and M is for Memory."[92]

In NSCLC, several groups have evaluated the prognostic impact of TILs, but only our group has focused on an Immunoscore assessment (low, intermediate, or high density of stromal $CD8^+$ T cells) and TNM-I.[77] Using principally the same strategy as the French research group used for colorectal cancer,[21] we were able to demonstrate a solid prognostic impact of the Immunoscore within each examined NSCLC pStage (IA, IB, IIA, IIB, and IIIA [Fig. 4]).[20,77] In the multivariate analysis of this study, $CD8^+$ T cell density had a prognostic impact

Percentage 5-year disease specific survival rates according to TNM and Immunoscore

TNM-I		Immunoscore (stromal CD8)		
		Low	Intermediate	High
pStage	IA	71	75	87
	IB	59	73	83
	IIA	51	58	61
	IIB	25	55	61
	IIIA	18	41	68

5-year DSS rates
≤ 40 %
41-59 %
60-80 %
> 80 %

Figure 4. From tumor, node, and metastasis (TNM) to TNM-Immunoscore (TNM-I) in non-small cell lung cancer? The American Joint Committee of Cancer and the Union for International Cancer Control define and regularly update the TNM classification systems. The current seventh edition of the lung cancer staging system, which was based on an initiative by the International Association for the Study of Lung Cancer, was first published in 2009 and took effect in January 2010.[93] When Donnem et al. assessed the association between Immunoscore (low, intermediate, and high density of stromal $CD8^+$ T cells) and pathological stage (pStage) (IA, IB, IIA, IIB, and IIIA), Immunoscore distinctly discriminated patients in two or three prognostic groups within each TNM pStage.[77] Within the respective pStages IIB and IIIA, in particular, there were substantial differences in prognosis when assessed by Immunoscore. Data from Donnem et al.[20] and Donnem et al.[77]

equal to that of NSCLC pStage, which significantly enhanced the discriminatory prognostic prediction. Presently, there is a national initiative in Norway for a prospective study validating an Immunoscore in NSCLC.[20] However, there are still several challenges regarding establishment of an Immunoscore, such as standardizing (1) immunohistochemical staining procedures, (2) the scoring system, and (3) the localization for scoring. According to our experience and results from the literature (see Table 1), stromal scoring of $CD8^+$ T cells appears superior to scoring in the tumor epithelial compartment.[88] But where in the tumor stromal areas should the $CD8^+$ cell assessment be performed (in the stroma in general or at the invasive margin)? Recent data from Donnem et al. indicate that the strongest prognostic impact of stromal scoring was at the invasive tumor margin, although verification is warranted.[77]

Currently, there are national and international initiatives to include assessments of TIL infiltration in colorectal cancer, breast cancer, and NSCLC as an adjunct to the TNM classification to improve patients' diagnoses, therapy, and prognosis.[20,89,94]

Immunoscore and Immunotherapies

The host immune system may contribute to both the development of cancer and the efficacy of cancer therapies, in particular where the induced tumor cell death is considered "immunogenic." The field of immunotherapy has had a renaissance in recent years, especially owing to the remarkable clinical efficacy observed after therapy with immune checkpoint inhibitors against various cancers, including NSCLC.[95–99] Recently, Kim et al. demonstrated that expression of programmed death ligand 1 (PD-L1) on pulmonary squamous cell carcinoma cells was significantly associated with increased $CD8^+$ TILs, which again was correlated with increased programmed death 1–positive ($PD\text{-}1^+$) TILs in pulmonary squamous carcinoma.[82] It has further been shown, that the efficacy of immune checkpoint blockade depends on TILs infiltrating and acting in the tumor tissues. It has been proposed that future strategies must be aimed at increasing $CD8^+$ TILs in number and functionality while minimizing their impairment by the immunosuppressive environment.[100]

There are currently efforts to predict which patients will respond to therapy with checkpoint blockade involving, in particular, anti–PD-1 and anti–PD-L1.[101] Here the immune contexture and the Immunoscore may allow prediction of response to immune-related therapies.[102–105]

Conclusion

In lung cancer development, neoplastic cells interact with their microenvironment, in which local cancer antigen-specific immune responses decide the destiny of the cancer cells. In recent years, there has been an increasing awareness of the immune contexture of NSCLC. This is defined according to immune cell type, density, and location, as well as according to the functional orientation of the involved immune cell populations in situ in the NSCLC microenvironment. The immune contexture represents, in fact, a major player in the development and fate of malignant disease. Elements of this immunogenic microenvironment and, in particular, the presence of TLSs correlate with survival. The possibility of assessing and evaluating a tumor's immune contexture will be a pivotal instrument to identify subsets of patients at high risk for relapse in order to modify therapy or allocate patients for adjuvant treatment. An established standardized immunopathological assessment, such as an Immunoscore added to the TNM classification (TNM-I), may be fundamental to offering a highly improved prognostic, and most possibly, also a predictive tool. A TNM-I for NSCLC will need to be evaluated and validated in large prospective studies before implementation. There are already proposals for

revision of the forthcoming eighth edition of the TNM classification for lung cancer.[106] The immune contexture should be incorporated into future editions of the TNM classification, as research has demonstrated substantial room for improvements regarding prognostic and possibly also predictive value. As outlined earlier in this article, the Immunoscore can delineate profound variety in NSCLC survival within each pStage. In this regard, Immunoscore should be further investigated in NSCLC and definitely evaluated for future TNM-I classifications. A score involving assessment of in situ immunity will also be fundamental for optimization of novel therapies such as immune checkpoint blockade, as it is essential to select patients who will respond to this treatment modality.[104]

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