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Review Article

Targeting tumor microenvironment for non-small cell lung cancer immunotherapy

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ABSTRACT

The tumor microenvironment (TME) is composed of different cellular and non-cellular elements. Constant interactions between tumor cells and the TME are responsible for tumor initiation, tumor progression, and responses to therapies. Immune cells in the TME can be classified into two broad categories, namely adaptive and innate immunity. Targeting these immune cells has attracted substantial research and clinical interest. Current research focuses on identifying key molecular players and developing targeted therapies. These approaches may offer more efficient ways of treating different cancers. In this review, we explore the heterogeneity of the TME in non-small cell lung cancer, summarize progress made in targeting the TME in preclinical and clinical studies, discuss the potential predictive value of the TME in immunotherapy, and highlight the promising effects of bispecific antibodies in the era of immunotherapy.

Introduction

Targeting the genetic alterations has contributed greatly to the treatment of non-small cell lung cancer (NSCLC).[1,2] Increasing evidence indicates that the tumor microenvironment (TME) plays a pivotal role in tumorigenesis and treatment responses.[3,4] The TME is complex and is composed of multiple cell types. Briefly, the TME includes the surrounding tumor vasculature, endothelial cells, fibroblasts, immune cells, and extracellular components.[5] These components form an interactive cellular- and non-cellular-based network during tumor initiation, progression, and therapeutic responses.

Most research on TMEs has focused on innate and adaptive immune cells.[6] Among the different kinds of adaptive immune cells, the roles of cytotoxic T lymphocytes in the TME have been studied most extensively.[7] Current research mostly focuses on this subset owing to its cytotoxic ability. Based on the current understanding of the innate immune system, it is evident that the innate immune response is pivotal for tumor progression. Innate immune cells, especially myeloid cells, consist of antitumoral or protumoral cells such as tumor-associated macrophages (TAMs), dendritic cells (DCs), and myeloid-derived suppressor cells (MDSCs).[8,9] The function and heterogeneity of each subset are discussed below [Fig. 1].

Immunohistochemistry (IHC) and flow cytometry are frequently used to detect components of the TME. However, these technologies do not comprehensively reflect the complexity of the TME, and NSCLC is a highly heterogeneous disease.[10] A deeper understanding of the TME can be achieved using highly developed technologies, such as single-cell RNA-sequencing (scRNA-seq),[11] mass cytometry,[12] and spatial transcriptomics.[13,14] The TME of NSCLC has been systematically analyzed at single-cell resolution in several recent studies. In the next section, we discuss the heterogeneity of the tumor immune microenvironment in NSCLC.

TME of NSCLC

Adaptive immune subsets

$CD8^+$ T cells

T cells in the TME experience chronic antigen stimulation, which leads to $CD8^+$ T cell exhaustion. These cells express high levels of immune-checkpoint proteins, including programmed death-1 (PD-1), T cell immunoglobulin and mucin domain-containing protein 3 (TIM-3), lymphocyte-activation gene 3 (LAG-3), and cytotoxic T-lymphocyte-associated protein 4 (CTLA-4),[15] which hinder the cytotoxic effects of $CD8^+$ T cells. However, T cell exhaustion is not a fixed state. A subset of progenitor exhausted cells has been identified in melanoma. This subset can differentiate into “terminally exhausted” tumor-infiltrating lymphocytes (TILs) and respond to anti-PD-1 therapy.[16] Interestingly, Guo et al[17] identified two $CD8^+$ T cell clusters that probably precede exhaustion based on scRNA-seq data from 12,346 T cells from 14 treatment-

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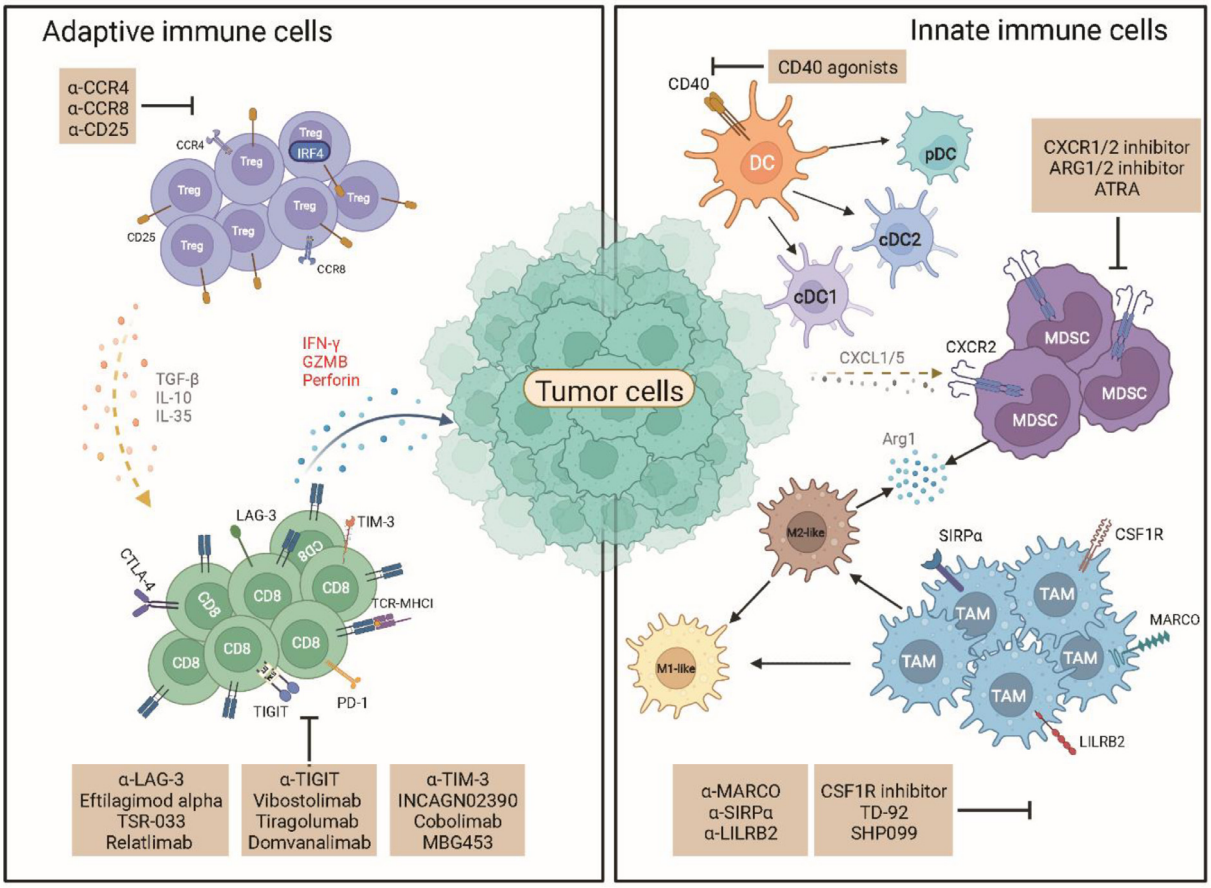

Fig. 1. The adaptive and innate immune cells in the tumor microenvironment. The left part displays the adaptive immune cells, which include primarily $CD8^+$ T cells and Tregs. $CD8^+$ T cells release cytotoxic chemokines to kill tumor cells. However, $CD8^+$ T cell expresses several immune checkpoints upon chronic antigen stimulation including PD-1, TIM-3, LAG-3, and TIGIT. This restricts the cytotoxic effect of $CD8^+$ T cells. Treg was an immunosuppressive subset that could release IL-10 and TGF-β to suppress the function of T cells. The right part displays the innate immune cells. Macrophages, dendritic cells, and MDSC are major subsets of innate immune cells. These cells consist of both antitumor immune cells and pro-tumor immune cells. Macrophages and dendritic cells could be divided into subtypes based on specific markers and functions. MDSC produces Arg1 and iNOS to suppress the function of T cells. These innate immune cells could be targeted or reprogramed to support antitumor immunity. Arg1: Arginase 1; ATRA: All-trans retinoic acid; CCR4: C-C chemokine receptor type 4; CCR5: C-C chemokine receptor type 5; CCR8: C-C chemokine receptor type 8; cDC1: Type 1 conventional dendritic cells; cDC2: Type 2 conventional dendritic cells; CSF-1R: Colony stimulating factor 1 receptor; CTLA-4: Cytotoxic T-lymphocyte-associated protein 4; CXCL1/5: C-X-C motif chemokine ligand 1/5; CXCR1/2: C-X-C motif chemokine receptor 1/2; DC: Dendritic cell; GZMB: Granzyme B; IFN-γ: Interferon gamma; IL: Interleukin; IRF4: Interferon regulatory factor 4; LAG-3: Lymphocyte-activation gene 3; LILRB2: Leukocyte immunoglobulin-like receptor subfamily B member 2; MARCO: Macrophage receptor with collagenous structure; MDSC: Myeloid-derived suppressor cells; MHC I: Major histocompability complex I; PD-1: Programed death-1; pDC: Plasmacytoid DC; SHP-2: Src homology-2 domain-containing protein tyrosine phosphatase-2; SIRPα: Signal regulatory protein α; TAM: Tumor- associated macrophages; TCR: T cell receptor; TGF-β: Transforming growth factor beta; TIGIT: T cell immunoreceptor with immunoglobulin (Ig) and ITIM domains; TIM-3: T cell immunoglobulin and mucin domain-containing protein 3; Treg: Regulatory T cells.

naïve patients with NSCLC. The ratio of "pre-exhausted" to exhausted T cells was used to discern a favorable prognosis for a subset of patients with lung adenocarcinoma (LUAD). However, the association between "terminally exhausted" TILs and "pre-exhausted" T-cells requires further investigation. Recent findings by Liu et al[18] revealed increased levels of precursor exhausted T cells (Texp) from responsive tumors after anti-PD-1-based therapies, which were absent in non-responsive tumors. The expansion of Texp cells probably results from: (1) local expansion and (2) replenishment by peripheral T cells. The results of a previous study highlight the role of precursor exhausted T cells in treatment responses and expand upon the concept of "clonal replacement".[19] In another study, a new subset of $CD8^+$ T cells expressing CD45RO, eomesodermin (EOMES), FAS, Ki67, LAG-3, PD-1, and TIM-3 were termed burned-out $CD8^+$ T cells (Ebo). Ebo $CD8^+$ T cells share several features with exhausted T cells, but are highly proliferative, representing the overactive and apoptotic phenotype of these cells. The accumulation of Ebo $CD8^+$ T cells in patients with advanced NSCLC is associated with resistance to anti-PD1 therapy.[20] The reinvigoration of exhausted $CD8^+$ T cells is the aim of immunotherapy. However, T cell differentiation is complex, and heterogeneity exists among different T cell subsets, as described above. Aging has also been reported to impair the expression of cytolytic molecules in $CD8^+$ T cells.[21] Thus, a comprehensive understanding of tumor-infiltrating T cells is urgently needed.

Regulatory T cells (Tregs)

Tregs display robust immunosuppressive function in CD4-positive ($CD4^+$) T cells, which were first found to maintain immunological self-tolerance and homeostasis.[22] Tregs are defined as $CD25^+$ forkhead box protein 3 $(FOXP3)^+$ cells based on analysis of their surface markers.[23] Tregs are frequently found in the TME and can be classified into different groups based on their distinct features. Three groups of tumor-associated Tregs have been identified previously, namely tumor-resident Tregs, tissue-resident Tregs, and circulating Tregs.[24–26] Tumor-associated Tregs account for 10–50% of $CD4^+$ T cells in tumors, which is profoundly higher than that found in peripheral blood from healthy individuals (2–5%). The immunosuppressive effect of Tregs involves the

release of immunosuppressive cytokines, including transforming growth factor β (TGF-β), interleukin 10 (IL-10), and IL-35.[27,28] Increased Treg infiltration influences invasion, metastasis, recurrence, drug responses, and the prognosis of patients with multiple cancers by inhibiting the cytotoxic role of $CD8^+$ T cells.[28] The prevalence of Tregs in the TME indicates that targeting Tregs may improve the efficacy of NSCLC treatment. This strategy for targeting Tregs is discussed in the next section.

Innate immune cells

DCs

Myeloid cells comprise both antitumor and immunosuppressive cells.[29] DCs are the most common antigen-presenting myeloid cells.[30] DCs can be classified into at least three subsets, based on specific markers and functions, including plasmacytoid DCs (pDCs), conventional type 1 DCs (cDC1s), and conventional type 2 DCs (cDC2s).[31] Among these subtypes, cDC1 plays a pivotal role in initiating T cell responses and is associated with survival in patients with cancer.[32]. Leader et al[33] profiled 361,929 cells from 35 early stage NSCLC lesions, and 49 immune clusters were identified. DCs are mainly composed of cDC1s, cDC2s, mature DCs enriched in regulatory molecules (mregDCs), and DC3s. DC3 is the most prevalent DC subtype found in tumors and is characterized as $CD14^+/CD163^+$.[33] In another study, Zilionis et al[34] identified four subsets of DCs in lung cancer: human pDC and hDC1–3. The hDC1 and hDC2 subsets mapped well to the cDC1 and cDC2 populations. hDC3 displayed an "activated" DC phenotype.[34] Both studies identified four DC clusters. The cDC1 and cDC2 populations were identified in accordance with the previous study.[35] However, disparate definitions exist for distinct subtypes, which may reflect differences in the analytical methods used and the markers selected.

Macrophages

In the study by Leader et al,[33] the distributions of different types of macrophages were analyzed simultaneously. Based on the expression of marker genes, macrophages can be classified into alveolar macrophages, interstitial macrophages, monocyte-derived macrophages, and azurocidin 1 $(AZU1)^+$ macrophages. Alveolar macrophages are the predominant subset in the TME, whereas interstitial macrophages are depleted. Zilionis et al[34] defined nine transcriptional states in human macrophages, which were classified as $hM\phi_{1-9}$ cells. Macrophages can be classified as M1 macrophages (with a pro-inflammatory phenotype) and M2 macrophages (with an anti-inflammatory phenotype), based on the expression of specific markers.[36,37] Among the nine subsets of macrophages mentioned above, no unique M1 phenotype macrophages were found. The M2 gene-expression signature was found with the $hM\phi_3$, $hM\phi_6$, and $hM\phi_8$ subsets.[34] These data suggest that immunosuppressive M2 macrophages occupy the TME. Targeting this subset of macrophages may improve treatment efficacies.

MDSCs

MDSCs represent another type of immunosuppressive myeloid cells in the TME.[38,39] MDSCs can be further divided into polymorphonuclear MDSCs (PMN-MDSCs) and monocytic MDSCs (M-MDSCs).[40] Infiltrating MDSCs can suppress the function of T cells through various mechanisms, such as programed death-ligand 1 (PD-L1) expression [41,42] and the secretion of immunosuppressive cytokines such as IL-10 and TGF-β.[43–45] Previously, MDSCs and inflammatory factors were profiled in the peripheral blood and resectable tumors of 49 patients with NSCLC. More C-C chemokine receptor type 5 (CCR5)-expressing circulating M-MDSCs were found in the peripheral blood of patients with lung cancer than in that from healthy donors. The frequency of M- and PMN-MDSCs was higher in tumor tissues than in the peripheral blood.[46] Dysregulated MDSC differentiation and chemoattraction may contribute to a higher abundance of MDSCs in the TME. The results of a previous study showed that higher levels of $CD45^+$ erythroid progenitor cells (EPCs, $CD71^+TER119^+$ cells) were found in anemic patients with cancer.[47] The same study revealed that $CD45^+$ EPCs could transdifferentiate into erythroid–myeloid hybrid cell (EDMC) populations. This process was dependent on granulocyte-macrophage colony-stimulating factor produced by tumor cells. EDMCs displayed robust immunosuppressive activity and reduced efficacy with anti-PD-1/PD-L1 treatment.[48] Thus, targeting the differential chemotaxis of MDSCs may prevent MDSC infiltration and improve immunotherapy.

As discussed above, scRNA-seq provides a high-resolution map of the TME. Immune cells can be subdivided into clusters for more precise characterization. Such information can help identify specific immune subsets that influence tumor progression or treatment responses, as well as potential targets.

Targeting TME to improve immunotherapy against NSCLC

Immune cells inside the TME can be identified using specific markers, including chemokine receptors, immunosuppressive molecules, and functional markers. The corresponding immune cells can be depleted or inhibited by targeting these markers with antibodies or inhibitors, respectively. Some molecules have entered clinical trials and achieved great success. In this section, we discuss the potential for targeting these immune cells in the TME [Tables 1 and 2].

Targeting immune checkpoints

Immune checkpoint inhibitors have achieved great success in treating NSCLC. Well-established immune checkpoint inhibitors, including anti-PD-1 and anti-CTLA-4 agents, have been approved for monotherapy and combination therapy (chemotherapy and immunotherapy).[49–56] In-depth research in the field of immuno-oncology has identified several novel immune checkpoints, including T cell immunoreceptor with immunoglobulin (Ig) and ITIM domains (TIGIT), TIM-3, and LAG-3. The corresponding inhibitors have been developed and entered into clinical trials.

TIGIT

TIGIT belongs to the poliovirus receptor/nectin family. TIGIT is expressed by various types of immune cells, including effector $CD8^+$ T cells, natural killer (NK) cells, and regulatory $CD4^+$ T cells.[57] TIGIT suppresses antitumor immunity by inhibiting the antitumor effects of $CD8^+$ T cells and NK cells.[58] In a bilaterally established model of LUAD, triple therapy with RadScopal + anti-TIGIT + anti-PD1 suppressed the growth of both primary and secondary tumors and significantly prolonged survival.[59] Vibostolimab is an anti-TIGIT antibody. A phase I study (NCT02964013) was conducted to investigate the safety and efficacy of vibostolimab in treating advanced solid tumors. Monotherapy or vibostolimab plus pembrolizumab was well-tolerated. In anti-PD-1/PD-L1-naïve NSCLC, combination therapy achieved 26% of the confirmed objective response rate (ORR); in anti-PD-1/PD-L1-refractory NSCLC, the ORRs following monotherapy and combination therapy were both 3%. These results demonstrate that vibostolimab exhibited antitumor activity in advanced solid tumors.[60] Recently, the results of the phase II CITYSCAPE trial (NCT03563716) were released. Combined treatment with the TIGIT inhibitor, tiragolumab, and atezolizumab achieved an ORR of 37%, compared with an ORR of 21% in NSCLC patients administered atezolizumab monotherapy. The ORR of the PD-L1 high-expression subgroup in patients receiving combination treatment was 66%. These results highlight the potential role of tiragolumab in combination with the anti-PD-L1 antibody.[61] Regarding ongoing trials, anti-PD-1 (zimberelimab) in combination with anti-TIGIT (domvanalimab) was launched in 30 previously treated patients with NSCLC (NCT04791839).

TIM-3

TIM-3 serves as a co-inhibitory receptor that primarily inhibits T-cell function.[62] INCAGN02390 is a novel antibody targeting TIM-3.

Table 1
Preclinical studies that target the tumor microenvironment.

Immune cell(s)	Agent(s)	Target(s)	Model(s)	Effect(s)	Reference(s)
Tregs	Docetaxel	NA	*In vitro*	Docetaxel-based chemotherapy decreased Treg after four cycles of treatment	67
	Anti-CD25 antibody	CD25	LLC model	Deplete Tregs; inhibit tumor growth when combined with radiation; increase the effector T cells	68
	Anti-CD25 antibody	CD25	MC38 and EO771 model; LL/2 and 4T1 model	Deplete Tregs; the antitumor effect was associated with $CD8^+$ T and NK cell infiltration level	69
	Nb-Fc1B0 (CCR8 antibody)	CCR8	LLC-OVA model	Deplete $CCR8^+$ Tregs; inhibit tumor growth when combined with anti-PD1	70
DCs	Anti-CD40 agonist antibody	CD40	LLC model	Induce E-$cadherin^+$ inflammatory DCs; enhance antitumor immunity; limit the tumor growth	76
Macrophages	Targeting specific markers on marcophages				
	Anti-MARCO antibody	MARCO	*In vitro*	Recover cytolytic activity of NK cells and T cells	78
	TD-92	CSF-1R	LLC model	Decrease the expression of CSF-1R on macrophages; inhibit tumor growth combined with PD-1	79
	CSF-1R inhibitor	CSF-1R	$Kras^{G12D/+}$; $p53^{-/-}$ NSCLC model	Improve the efficacy of anti-angiogenic blockade and ICB	81
	SIRPα-Fc	SIRPα/CD47 signaling	LLC model	Sensitize the tumor to VEGF blockade	83
	Reprogramming of macrophages				
	SHP099	SHP-2	344SQ NSCLC adenocarcinoma	Repolarization of M2 TAMs to the antitumor M1 phenotype; triple therapy with radiation and anti-PD-L1 significantly inhibits the primary tumor growth	85
	Anti-LILRB2 antibody	LILRB2	LLC model	Enhance TNF-α release; combination of PD-L1 and LILRB2 significantly reduces tumor size	88
	Anlotinib	VEGFR, FGFR, PDGFR, and c-kit	LLC model	Expand M1-like TAM; inhibit tumor growth when combined with αPD-1	87
MDSCs	CXCR1/2 inhibition	CXCR1/2	KP mouse model	Deplete the subsets of gMDSCs; combination with SHP099 further inhibits tumor growth with anti-PD-1	92
	ATRA	NA	LKB1-deficient murine models	Suppress the percentage and function of gMDSCs; inhibit tumor growth when combined with anti-PD-1	93
	ARG1/2 inhibitor	ARG1	$Kras^{G12D}$ lung cancer model	Inhibit the function of MDSC; inhibit tumor growth and enhance T cell numbers and function	94

ARG1: Arginase 1; ATRA: All-trans retinoic acid; CCR8: C-C chemokine receptor type 8; CSF-1R: Colony-stimulating factor 1 receptor; CXCR1/2: C-X-C motif chemokine receptor 1/2; DC: Dendritic cells; FGFR: Fibroblast growth factor receptor; gMDSC: Granulocytic MDSC; ICB: Immune checkpoint blockade; KP: $Kras^{G12D/+}$ and $p53^{-/-}$; LKB1: Liver kinase B1; LILRB2: Leukocyte immunoglobulin-like receptor subfamily B member 2; LLC: Lewis lung cancer; LLC-OVA: LLC-ovalbumin; LILRB2: Leukocyte immunoglobulin-like receptor subfamily B member 2; MARCO: Macrophage receptor with collagenous structure; MDSC: Myeloid-derived suppressor cells; NA: Not available; NK: Natural killer; NSCLC: Non-small cell lung cancer; PD-1: Programed death-1; PDGFR: Platelet-derived growth factor receptor; PD-L1: Programmed death-ligand 1; SHP-2: Src homology-2 domain-containing protein tyrosine phosphatase-2; SIRPα: Signal regulatory protein α; TAMs: Tumor-associated macrophages; TNF-α: Tumor necrosis factor-α; Tregs: Regulatory T cells; VEGF: Vascular endothelial growth factor; VEGFR: Vascular endothelial growth factor receptor.

The results of a preclinical study demonstrated that interferon gamma (IFN-γ) production increased following treatment with INCAGN02390. A phase I study of INCAGN02390 is ongoing for patients with advanced solid tumors (NCT03652077). The efficacy of combinations containing anti-TIM-3 (cobalolimab), anti-PD-1 (dostarlimab) and docetaxel-based chemotherapy is under observation in clinical trial NCT04655976. Another trial is being conducted to explore the efficacy of combination therapy of anti-PD-1 and anti-TIM3 with platinum-based doublet chemotherapy (NCT03307785). The preliminary results of combination therapy have been reported in a phase II dose-expansion trial (NCT02608268). In that study, 16 patients with melanoma and 17 patients with NSCLC were enrolled and treated with antibodies against TIM-3 (MBG453) and PD-1 (spartalizumab). Combination therapy is safe, but its efficacy is limited.[63]

LAG3

LAG-3 is a novel inhibitory coreceptor that can inhibit T-cell activation and function.[64] Eftilagimod alpha, a soluble LAG-3 protein, directly targets LAG-3. Related clinical trials have been conducted for metastatic melanoma,[65] metastatic breast cancer,[66] and NSCLC (NCT03625323). TSR-033 is a LAG-3 antibody. A phase 1 study of TSR-033, alone or in combination with anti-PD-1, is ongoing for patients with advanced solid tumors (NCT03250832). Another anti-LAG-3 antibody, relatlimab, is under investigation in different clinical trials (NCT04623775 and NCT04205552).

Targeting Tregs

Depleting Tregs is an appropriate approach for enhancing the therapeutic efficacy of immunotherapy, and docetaxel is widely used in NSCLC treatment. Four cycles of docetaxel treatment decreased the levels of Tregs. *In vitro* experiments confirmed these results.[67] CD25 is a surface marker of Tregs. Targeting CD25 with a specific antibody efficiently depleted Tregs and inhibited tumor regression. In Lewis lung cancer (LLC) models, low-dose radiotherapy and anti-CD25 antibodies efficiently depleted Tregs and increased the levels of effector T cells. In addition, the growth of irradiated and distal non-irradiated tumor cells was significantly inhibited.[68] In this model, anti-CD25 treatment alone did not efficiently inhibit tumor growth. This finding is in accordance with the results of the following discussed study. Anti-CD25 was effective in MC38 and EO771 mouse models, whereas minimal effects were observed in LL/2 and 4T1 mouse models. This discrepancy was

Table 2
Clinical trials targeting the tumor microenvironment.

Target(s)	Agent(s)	Combination	Phase	NCT number	Condition(s)	Status	Result(s)
TIGIT	Vibostolimab	Anti-PD-1 (pembrolizumab)	I	NCT02964013	Advanced solid tumors (NSCLC)	Active, not recruiting	NA
	Tiragolumab	Anti-PD-L1 (atezolizumab)	II	NCT03563716	NSCLC	Active, not recruiting	The combination therapy achieved an ORR of 37%
	Domvanalimab	Anti-PD-1 (zimberelimab)	II	NCT04791839	NSCLC	Recruiting	NA
TIM-3	Eftilagimod alpha	Anti-PD-1 (pembrolizumab)	II	NCT03625323	NSCLC	Active, not recruiting	NA
	TSR-033	Anti-PD-1 (dostarlimab)/docetaxel	I	NCT03250832	Advanced solid tumors (NSCLC)	Active, not recruiting	NA
	Relatlimab	Anti-PD-1 (nivolumab)	II	NCT04623775	NSCLC	Recruiting	NA
	Relatlimab	Anti-PD-1 (nivolumab)	II	NCT04205552	NSCLC	Recruiting	NA
LAG-3	TSR-022	Anti-PD-1 (TSR-042)/carboplatin-pemetrexed	I	NCT03307785	Advanced cancer (NSCLC)	Active, not recruiting	NA
	INCAGN02390	NA	I	NCT03652077	Advanced malignancies (NSCLC)	Completed	NA
	Cobolimab	Anti-PD-1 (dostarlimab)/docetaxel	II/III	NCT04655976	Advanced NSCLC	Recruiting	NA
	MBG453	Anti-PD-1 (PDR001)	I/II	NCT02608268	Advanced malignancies (NSCLC)	Active, not recruiting	NA
CCR4	Mogamulizumab	NA	I	NCT01929486	Advanced or recurrent cancer	Unknown	Well-tolerated but induces limited clinical efficacy
	Mogamulizumab	Docetaxel	I	NCT02358473	NSCLC	Completed	NA
	Mogamulizumab	Nivolumab	I	NCT02476123	Advanced solid tumors (NSCLC)	Completed	Acceptable safety profile and antitumor activity
	Mogamulizumab	Anti-PD-L1 (durvalumab)/anti-CTLA-4 (tremelimumab)	I	NCT02301130	Advanced solid tumors (NSCLC)	Completed	Acceptable safety profile, but did not result in potent antitumor efficacy
CSF-1R	Cabiralizumab	CD40 agonist (APX005M)/anti-PD-1 (nivolumab)	I	NCT03502330	NSCLC	Recruiting	NA
Arg1	INCB001158	Anti-PD-1 (pembrolizumab)	I/II	NCT02903914	Advanced/metastatic solid tumors	Active, not recruiting	Well tolerated and showed responses

Arg1: Arginase 1; CCR4: C-C chemokine receptor type 4; CSF-1R: Colony-stimulating factor 1 receptor; CTLA-4: Cytotoxic T-lymphocyte-associated protein 4; LAG-3: Lymphocyte-activation gene 3; NA: Not available; NSCLC: Non-small cell lung cancer; PD-1: Programmed death-1; PD-L1: Programmed death-ligand 1; TIGIT: T cell immunoreceptor with immunoglobulin (Ig) and ITIM domains; TIM-3: T cell immunoglobulin and mucin domain-containing protein 3.

attributed to the differences in the infiltration levels of $CD8^+$ T and NK cells.[69] These findings may suggest that the depletion of Tregs alone is insufficient for eliminating tumors and that cytotoxic cells are essential for tumor control.

C-C chemokine receptor type 8 (CCR8) is expressed in a subset of Tregs and CCR8 expression correlates with more advanced diseases.[26] Single-cell analysis of the T cell compartment in LLC-ovalbumin (LLC-OVA) tumors revealed distinct CCR8 expression in Tregs. Nb-Fc1B is an antibody that blocks CCR8, along with antibody-dependent cell-mediated cytotoxicity (ADCC). Treating LLC-OVA tumor-bearing mice with Nb-Fc1B successfully depleted $CCR8^+$ Tregs in an NK cell-dependent manner. When combined with anti-PD1, tumor growth was largely inhibited.[70] C-C chemokine receptor type 4 (CCR4) is highly expressed on the surface of inhibitory Tregs. KW-0761 is a CCR4 monoclonal antibody that can deplete $CCR4^+$ Tregs via ADCC. In patients with lung cancer, even low-dose KW-0761 can deplete Tregs. Monotherapy with KW-0761 (mogamulizumab) is safe and well-tolerated.[71] Clinical trials are ongoing to test the efficacy of mogamulizumab combined with anti-PD1/anti-PD-L1. In the phase I clinical trial NCT02476123, combined mogamulizumab and nivolumab treatment was safe and well tolerated. The antitumor effect was moderate in multiple types of tumors. For NSCLC, three partial responses and three disease stabilizations were observed in 15 patients.[72] In another phase I/II study, 114 patients diagnosed with locally advanced or metastatic solid tumors were enrolled. Patients were treated with mogamulizumab (1 mg/kg) plus nivolumab (240 mg). These doses were acceptable and tolerable. However, no additional effect was observed with combination therapy, and only a 10.5% ORR was observed.[73] In another study (NCT02301130), mogamulizumab was combined with durvalumab. However, the ORR was only 5.3% with the combination treatment.[74]

Targeting DCs

Targeting DC activation with different approaches provides therapeutic options. Several factors are required for the development and maturation of DCs, including FMS-like tyrosine kinase 3 ligand and CD40.[75] Targeting these factors may enhance the function of DCs. Anti-CD40 treatment induced E-$cadherin^+$ inflammatory DCs in the lungs of Rag1-knockout mice. The LLC model showed slower growth after the transfer of E-$cadherin^+$ DCs. The TME was reprogrammed with increased T helper 1 (Th1) and Th17 cell responses and reduced Treg cells. In addition, antitumor $CD103^+CD8^+$ T cells and tumor-specific $CD8^+$ T cell responses were also enhanced. This finding highlights the potential of using anti-CD40 to improve the efficiency of immunotherapy in patients.[76]

Targeting macrophages

Targeting specific markers on macrophages

Fleur et al[77] observed the expression levels of scavenger receptors and macrophage receptor with collagenous structure (MARCO) in a distinct subpopulation of TAMs in a cohort of 352 patients with NSCLC. This cluster of macrophages was located close to the tumor cell nests. Co-expression of PD-L1 was observed in $MARCO^+$ macrophages. $MARCO^+$

macrophages suppress the activity of cytotoxic T and NK cells. Targeting MARCO with a specific antibody reversed the effect of MARCO$^+$ macrophages, resulting in the recovery of the cytolytic activities of NK and T cells.[78] TD-92 is a novel erlotinib derivative. Combined anti-PD-1 and TD-92 treatment significantly reduced tumor growth and increased survival in a model of LLC. Analysis of the microenvironment revealed a significant decline in pro-tumorigenic CD11b$^+$F4/80$^+$ macrophages. Mechanistically, TD-92 can decrease the expression of colony-stimulating factor 1 receptor (CSF-1R) in macrophages.[79] Using a genetically engineered mouse model of Kras$^{G12D/+}$; p53$^{-/-}$ NSCLC (KP model),[80] monocyte- and alveolar-origin TAMs were found to be associated with resistance to immune-checkpoint blockade (ICB). These subsets of macrophages facilitate the infiltration of PD-1$^+$ regulatory T cells. Combination treatment with the CSF-1R inhibitor and cisplatin depleted both subsets of macrophages, thereby improving the efficacy of anti-angiogenic blockade and immunotherapy.[81] The cluster of differentiation 47/signal-regulatory protein alpha (CD47/SIRPα) pathway could inhibit phagocytosis, which inhibits the function of macrophages.[82] Recent data revealed that CD47 upregulation resulted in resistance to vascular endothelial growth factor/vascular endothelial growth factor receptor (VEGF/VEGFR) inhibitors. Blocking CD47 signaling with SIRPα-Fc sensitized tumors to VEGF blockade, and this effect was reversed by eliminating macrophages *in vivo*.[83] These findings suggest that blocking CD47/SIRPα signaling in macrophages could enhance phagocytosis and suppress tumor growth.

Reprograming macrophages

Macrophage repolarization has been shown to be effective in controlling tumor growth. SHP099 is a unique inhibitor that selectively blocks the activity of Src homology-2 domain-containing protein tyrosine phosphatase-2 (SHP-2),[84] which is widely expressed in immune cells. In a mouse model of anti-PD-1-resistant NSCLC, triple therapy with radiation, anti-PD-L1, and SHP099 significantly inhibited primary tumor growth. Abscopal effects were only observed with triple therapy. The observed effect was related to the repolarization of M2 TAMs to the antitumor M1 phenotype. When macrophages were depleted, the effect of triple therapy was diminished.[85] Anlotinib is an oral tyrosine-kinase receptor inhibitor approved for treating lung cancer as a third-line treatment.[86] In a mouse model of LLC, anlotinib treatment reduced the frequency of M2-like TAMs, whereas the abundance of the population of M1-like TAMs increased. When combined with αPD-1, tumor growth was significantly inhibited compared with either treatment alone.[87] TAMs isolated from patient biopsies ubiquitously express leukocyte immunoglobulin-like receptor subfamily B member 2 (LILRB2). *Ex vivo* treatment with an anti-LILRB2 antibody enhanced tumor necrosis factor (TNF)-α release, whereas lower levels of the cell-surface markers CD163, CD209, and CD14 were observed. These findings suggest that TAMs can be reprogramed by LILRB2 blockade. Furthermore, combined inhibition of PD-L1 and LILRB2 led to significantly reduced tumor sizes in LLC tumor-bearing mice,[88] indicating that LILRB2 is a potential target for treating lung cancer.

Clinical trials targeting macrophages have been initiated based on these preclinical studies. Recently, the efficiencies of the CD40 agonist, APX005M (sotigalimab), and the CSF-1R inhibitor, cabiralizumab, were tested in a phase I trial with or without nivolumab. The patients enrolled in the clinical trial had melanoma, kidney cancer, or NSCLC. The combination treatments were well tolerated, although the dosing frequency and sequence require further trials.[89]

Targeting MDSCs

Various researchers have reported different approaches for inhibiting MDSC infiltration or impacting MDSC function.[90] C-X-C motif chemokine receptor 2 (CXCR2) is a trafficking receptor expressed on MDSCs,[91] which can be attracted by C-X-C motif chemokine ligand 1/5 (CXCL1 and CXCL5). In a mouse model of KP, SHP2 inhibition promoted granulocytic MDSC (gMDSC) infiltration by elevating the expression levels of CXCL1 and CXCL5, which limits the use of SHP2 inhibitors. CXCR1/2 inhibition with an SHP2 inhibitor depleted subsets of gMDSCs, which further inhibited tumor growth.[92] These data suggest that inhibiting MDSC attraction could provide further benefits when SHP2 inhibitor is used. In liver kinase B1 (LKB1)-deficient lung cancer cells, the production of Glu-Leu-Arg (ELR)$^+$ CXC chemokines was significantly upregulated, which led to the local enrichment of gMDSCs within the TME. All-trans-retinoic acid (ATRA) suppressed the percentage and function of gMDSCs in LKB-1 mutant model. Treatment of tumor-bearing mice with ATAR can enhance the infiltration and function of CD8$^+$ T cells. When combined with anti-PD-1, tumor growth was efficiently inhibited.[93] Arginase 1 (Arg1) can contribute to the immunosuppressive function of MDSCs. Thus, inhibiting Arg1 may represent a new therapeutic strategy. *In vitro* treatment with an Arg1/2 inhibitor (compound 9) restored T cell function when co-cultured with MDSCs. In a model of Kras^{G12D} lung cancer, treatment with compound 9 inhibited tumor growth and enhanced the abundance and function of T cells.[94] INCB001158 is a novel arginase inhibitor. A phase I clinical trial was conducted for patients with advanced/metastatic solid tumors to evaluate the efficiency of INCB001158 alone or in combination with pembrolizumab.[63]

TME components as biomarkers for NSCLC immunotherapy

The complex functions of different immune subsets can influence the responses of patients with NSCLC to immunotherapy. Thus, these components can potentially be used to predict the progression and responses of patients. Blood or surgical tumor samples are frequently used to detect the presence and percentage of specific types of immune cells [Table 3].

Biomarkers in the peripheral blood

Multiple immune markers in the peripheral blood have been tested. Recent results showed that tumor-associated autoantibodies (TAAbs) could be used to predict the efficacy of immunotherapy in patients with advanced NSCLC.[95] Neutrophil-to-lymphocyte ratios (NLRs) have been used to predict the outcomes of ICB. High baseline NLRs have been linked to a worse overall survival (OS) and progression-free survival (PFS) in multiple types of tumors, including NSCLC.[96,97] An increased NLR after treatment has been associated with worse outcomes.[98] In another study, blood samples from 53 patients treated with nivolumab were analyzed to determine the abundances of gMDSCs, neutrophils, eosinophils, and lymphocytes. The results demonstrated that high baseline levels of gMDSCs, low absolute neutrophil counts, high eosinophil counts, and low NLRs predicted significant improvements in the OS and PFS.[99] CD4$^+$CD25$^+$CD127^{lo}FoxP3$^+$ Treg cells were associated with treatment responses in patients with advanced NSCLC administered anti-PD-1/PD-L1 therapy. The frequency of circulating Treg cells significantly decreased 7 days after treatment in the responder group.[100] However, the results of another study indicated that high frequencies of circulating Treg cells (CD4$^+$CD25$^+$CD45RA$^-$FoxP3$^+$) at one week after anti-PD-1 therapy correlated with a better response.[101] This discrepancy may reflect the different surface markers examined in both studies, and the lack of CD45RA expression may indirectly reflect an effective antitumor immune response.[102]

TME-related biomarkers

Tumor-infiltrating lymphocytes

TILs, especially CD8$^+$ T cells, have been associated with an improved prognosis.[103] Kim et al[104] demonstrated that PD-L1 expression was associated with increased TILs in pulmonary squamous carcinoma. Thus,

Table 3
Prognostic value of the tumor microenvironment.

Subset(s)	Marker(s)	Localization	Detection time	Detection method	Association with prognosis/response	Reference(s)
TAAbs	NA	P	Baseline	ELISA	Better prognosis	95
NLR	NA	P	Baseline	FACS	High NLR associated with worse survival	96,97
NLR	NA	P	After treatment	FACS	Increase associated with worse survival	98
TCRβ chains	Complementarity-determining region 3	P	Baseline/after treatment	TCRβ sequencing	High diversity/TCR clonality associated with better prognosis	111
Tregs	$CD4^+$ $CD25^+$ $CD127^{lo}$ $FoxP3^+$	P	7 days after treatment	FACS	Decrease in responder	100
Tregs	$CD4^+$ $CD25^+$ $CD45RA^-$ $FoxP3^+$	P	7 days after treatment	FACS	Increase associated with a better response	101
TILs	$PD\text{-}L1^+$ TIL^+	I	Baseline	IHC	Better prognosis	107
TILs	$PD\text{-}L1^-$ TIL^-	I	Baseline	IHC	Poor prognosis	107
TILs	$CD8^+$ $SP142^+$/$CD8A^+$ $CD274^+$	I	Baseline	IHC/RNA-seq	Better prognosis	108
Interferon-γ gene signature	CD8A, GZMA, GZMB, IFN-γ, EOMES, CXCL9, CXCL10, and TBX21	I	Baseline	RNA-seq	Better prognosis	112
Interferon-γ gene signature	T cell-inflamed GEP panel	I	Baseline	RNA-seq	Better prognosis	113
Tregs	IRF4	I	Baseline	RNA-seq	Poor prognosis	118
DCs	DC-LAMP	TLS	Baseline	IHC	Better prognosis	120
DCs	DC signature	I	Baseline	RNA-seq	Better prognosis	121
Macrophages	CD68/CD163	I	Baseline	IHC	Poor prognosis	122
Macrophages	SIRPα/CD68	I	Baseline	IHC	Poor prognosis	123
Macrophages	CD163/PD-1	I	Baseline	IHC	Poor prognosis	124
Macrophages	TREM2	I	Baseline	IHC	Poor prognosis	125
Plasma cells	Plasma cells signature	I	Baseline	RNA-seq	Better prognosis	126

CXCL9: C-X-C motif chemokine receptor 9; CXCL10: C-X-C motif chemokine receptor 10; DCs: Dendritic cells; DC-LAMP: Dendritic cell lysosomal associated membrane glycoprotein; ELISA: Enzyme-linked immunosorbent assay; EOMES: Eomesodermin; FACS: Fluorescence-activated cell sorting; FoxP3: Forkhead box P3; GEP: Gene expression profile; GZMA: Granzyme A; GZMB: Granzyme B; I: Intratumoral; IFN-γ: Interferon gamma; IHC: Immunohistochemistry; IRF4: Interferon regulatory factor 4; NA: Not available; NLR: Neutrophil-to-lymphocyte ratio; P: Peripheral blood; PD-1: Programed death-1; PD-L1: Programed death-ligand 1; RNA-seq: RNA-sequencing; SIRPα: Signal regulatory protein α; TAAbs: Tumor associated autoantibodies; TBX21: T-box protein 21; TCR: T cell receptor; TILs: Tumor-infiltrating lymphocytes; TLS: Tertiary lymphoid structures; Treg: Regulatory T cells; TREM2: Triggering receptor expressed on myeloid cells-2.

a new classification of TMEs based on TILs and PD-L1 levels was proposed. Patients can be classified into four types, namely type I ($PD\text{-}L1^+TIL^+$), type II ($PD\text{-}L1^-TIL^-$), type III ($PD\text{-}L1^+TIL^-$), and type IV ($PD\text{-}L1^-TIL^+$).[105,106] Previous analysis indicated that type-II patients had a very poor prognosis, whereas type-I had the best prognosis.[107] The combined predictive value of $CD8^+$ TIL levels and PD-L1 expression was tested in a cohort of 85 patients treated with nivolumab. Patients with high levels of $CD8^+$ TILs (CD8A) and PD-L1 (CD274), as determined either by IHC or messenger RNA (mRNA)-expression analysis, were classified as having a longer PFS.[108] As $CD8^+$ TILs are the most efficient cytotoxic cells, a higher CD8-to-CD3 ratio has been associated with longer PFS and OS in patients with NSCLC who received nivolumab.[109] Previously, a low incidence of PD-1 expression among $CD8^+$ cells was associated with a prolonged PFS.[109] An effective antitumor immune response requires T cell receptor (TCR) binding to the major histocompatibility complex/antigen short-peptide complex.[110] In a study, the complementarity-determining region 3 of TCR chains was sequenced as a predictive biomarker in patients with NSCLC who were treated with ICB. The results showed that high baseline $PD\text{-}1^+CD8^+$ TCR diversity and increased $PD\text{-}1^+CD8^+$ TCR clonality after ICB treatment were associated with a prolonged PFS.[111]

Successful antitumor immunity is dependent on effector chemokines, including IFN-γ. In a phase II study of NSCLC (POPLAR), a gene signature for effector T cells and IFN-γ was defined (CD8A, granzyme A [GZMA], granzyme B [GZMB], IFN-γ, EOMES, C-X-C motif chemokine receptor 9 [CXCL9], C-X-C motif chemokine receptor 10 [CXCL10], and T-Box transcription factor 21 [TBX21]). Patients with high expression levels of these genes displayed improved OS after atezolizumab treatment.[112] In another study, a pan-tumor T cell-inflamed panel (18 genes) was constructed, reflecting inflammatory IFN-γ responses and increasing the accuracy of prognosis for ICB treatment.[113] Based on weighted correlation-network analysis of CD8-associated genes, LUAD can be classified into two molecular subtypes. A 10-gene signature risk model was constructed, and patients with higher risk scores had lower survival rates.[114]

Evidence has shown that $PD\text{-}1^+$ regulatory T cells are associated with cancer hyperprogression following treatment with PD-1 inhibitors.[115] Additionally, the PD-1-expression balance between effector and regulatory T cells may serve as a biomarker for the effectiveness of PD-1 blockade therapy. Group R was proposed as the responder group, based on PD-1 expression. Group R was defined as having PD-1 positivity in ≥40% of $CD8^+$ TILs and a PD-1-expression ratio in $CD8^+$ T cells to Treg cells among TILs of ≥1. Patients in group R had a significantly longer PFS.[116] Interferon regulatory factor 4 (IRF4) is a transcription factor involved in T cell differentiation and metabolism.[117] IRF4 is specifically expressed by a subset of intratumoral $CD4^+$ effector Treg cells in patients with NSCLC, according to single-cell analysis of tumors, adjacent lung tissues, and blood samples from these patients. The abundance of IRF4 Tregs was correlated with a poor prognosis.[118]

DCs

DCs are indispensable for successful antitumor immunity. Mature DCs are characterized by the expression of dendritic cell lysosome-associated membrane glycoprotein (DC-LAMP).[119] In a retrospective analysis of 376 patients with NSCLC, the density of mature DCs correlated with a longer OS. This cluster of mature DCs was localized in tertiary lymphoid structures (TLSs). When combined with the stromal $CD8^+$ cell density, patients with the highest risk of death could be specifically identified.[120] In a cohort of patients with lung cancer, the DC signature could predict an improved survival benefit for

Table 4
Recent advances in bispecific antibodies in NSCLC.

Targets	BsAb	Status	Phase	NCT number	Conditions
PD-1/PD-L1	IBI318	Recruiting	I	NCT04777084	Advanced NSCLC
PD-1/LAG-3	MGD013	Active, not recruiting	I	NCT03219268	Advanced solid tumors (NSCLC)
PD-1/LAG-3	RO7247669	Recruiting	I	NCT04140500	Advanced and/or metastatic solid tumors (NSCLC)
PD-1/TIM-3	AZD7789	Recruiting	I/II	NCT04931654	Advanced or metastatic solid cancer (NSCLC)
PD-1/TIM-3	RO7121661	Active, not recruiting	I	NCT03708328	Advanced and/or metastatic solid tumors (NSCLC)
PD-1/CTLA-4	AK104	Recruiting	II	NCT05215067	Advanced NSCLC
PD-1/CTLA-4	MGD019	Recruiting	I	NCT03761017	Unresectable/metastatic cancer (NSCLC)
PD-1/VEGF	AK112	Recruiting	I/II	NCT04900363	NSCLC
PD-L1/CD27	CDX-527	Recruiting	I	NCT04440943	Advanced malignancies (NSCLC)
PD-L1/41BB	GEN1046	Recruiting	I/II	NCT03917381	Solid tumors (NSCLC)
PD-L1/41BB	INBRX-105	Recruiting	I	NCT03809624	Metastatic solid tumors (NSCLC)
CD47/PD-L1	PF-07257876	Recruiting	I	NCT04881045	Advanced tumors (NSCLC)
CD3/B7H4 a	GEN1047	Recruiting	I/II	NCT05180474	Malignant solid tumors (NSCLC)
TIGIT/PD-L1	HLX301	Recruiting	I/II	NCT05102214	Locally advanced or metastatic solid tumors (NSCLC)
CTLA-4/LAG-3	XmAb®22841	Recruiting	I	NCT03849469	Advanced solid tumors (NSCLC)

BsAb: Bispecific antibody; CD47: Cluster of differentiation 47; CTLA-4: Cytotoxic T-lymphocyte-associated protein 4; LAG-3: Lymphocyte-activation gene 3; NSCLC: Non-small cell lung cancer; PD-1: Programed death-1; PD-L1: Programed death-ligand 1; TIGIT: T cell immunoreceptor with immunoglobulin (Ig) and ITIM domains; TIM-3: T cell immunoglobulin and mucin domain-containing protein 3; VEGF: Vascular endothelial growth factor.

treatment with atezolizumab, but not docetaxel. Moreover, the study showed that the DC gene signature could predict survival for a patient subgroup with PD-L1$^+$ immune cells, but not for a PD-L1$^-$ subgroup.[121]

Macrophages

The prognostic value of M2 macrophages was examined in 349 patients with NSCLC. CD68 and CD163 expressions were detected to identify M2 macrophages. The results demonstrated that an elevated M2 ratio (CD163$^+$/CD68$^+$) predicted a poor OS.[122] In another cohort of 98 patients with NSCLC, the expression levels of CD47, SIRPα, and CD68 expression were detected. A high CD68$^+$ Mφ-score predicted an improved OS. However, SIRPα^+CD68$^+$ macrophages have been associated with a poor prognosis, indicating that SIRPα is a potential target.[123] The above-mentioned MARCO$^+$ macrophages predicted a weak trend toward worse survival, but did not reach statistical significance.[77] PD-1 is frequently expressed in activated T lymphocytes. However, PD-1 can also be expressed by TAMs. In 213 human LUADs, PD-1 was preferentially expressed by CD163$^+$ TAMs in the tumor stroma. Survival analysis showed that stromal PD-1$^+$ TAM infiltration predicted poor survival.[124] A subset of triggering receptor expressed on myeloid cells-2 (TREM2)$^+$ TAMs was identified based on data deposited in The Cancer Genome Atlas and single-cell sequencing datasets. In a cohort of 40 patients with NSCLC who received PD-1 based ICB treatment, the TREM2$^+$TAM^{hi} group showed poor survival.[125] These results may provide new biomarkers for determining the prognosis of patients receiving immunotherapy in clinical practice.

TLSs

The results of numerous studies have demonstrated that TLSs are associated with favorable survival across a wide range of cancers.[126,127] Recent data showed that B and plasma cell signals were associated with an OS benefit in patients treated with atezolizumab based on the transcriptomes of POPLAR and OAK (clinical trials of atezolizumab *vs.* chemotherapy in NSCLC). In addition, B cell, germinal center B cell, and plasma cell signatures were determined from scRNA-seq data. The results indicated a pivotal role of plasma cells in the OS benefit from atezolizumab.[126]

Perspective of the TME as target for NSCLC

Monoclonal antibodies targeting immune checkpoints elicit significant responses during NSCLC treatment.[128] However, the TME is complex, and the interaction between different pathways hinders the success of immunotherapy. In addition, the adverse effects of antibodies restrict their clinical use. The emergence of bispecific antibodies may expand the current applications of cancer immunotherapy.[129] In this section, we discuss the current bispecific antibodies that are being used in preclinical and clinical studies [Table 4].

Preclinical studies

MEDI5752 is a monovalent bispecific antibody that inhibits both PD-1 and CTLA4 in PD-1$^+$ activated T cells. *In vivo* experiments demonstrated that MEDI5752 accumulated in tumors at higher levels than an isotype-matched control monoclonal antibody. When 10 mg/kg MEDI5752 was administered to mice, the antitumor effect was strong, with 60% of the mice showing complete tumor clearance. This was superior to a single treatment using anti-PD-1 or anti-CTLA4 monoclonal antibodies.[130] In two patients with advanced gastric adenocarcinoma and clear cell carcinoma of the kidney, treatment with MEDI5752 achieved robust partial responses.[130] Bispecific anti-PD-L1/PD-1 antibodies have been reported in a patent (US2019010232). The antitumor effect was examined in a model of lung carcinoma. The v3.2 antibody delayed tumor growth significantly at a dose of 0.02 mg/kg, compared with the delay found with an anti-PD-L1 antibody, an anti-PD-1 antibody, or a combination of both.[131] A bispecific antibody targeting CD47 and CTLA4 was constructed, which preferentially depleted inducible T-cell co-stimulator high (ICOS^{hi}) immunosuppressive Treg cells in the TME. Treatment with the CD47/CTLA4 bispecific antibody significantly inhibited tumor growth in MC38 and CT26 models in a T cell-dependent manner.[132]

In addition to the dual inhibition of immune checkpoints, the simultaneous inhibition of immunosuppressive cells and promotion of systemic T cell responses is also a promising strategy. N809 is a bifunctional agent that contains an IL-15 superagonist fused with two αPD-L1 domains. In 4T1 and MC38 mouse models, treatment with N809 inhibited tumor growth and metastasis. The TME was characterized by enhanced NK and CD8$^+$ T cell activation and reduced Tregs, M-MDSCs, and M2-like macrophages after treatment.[133] Stimulation of CD3-mediated signaling has been used to treat hematologic malignancies. Based on these observations, a bispecific antibody that simultaneously targets CD3ϵ and PD-L1 was developed. PD-L1 $\times$ CD3 generated a superior antitumor effect in a mouse model than treatment with a single agent. Mechanistically, this effect was due to the blockade of PD-L1 on DCs,[134] in accor-

dance with a previous report showing that PD-L1 on DCs inhibits T cell activation.[135,136]

Clinical trials

Several bispecific antibodies have been investigated in clinical trials with promising results in preclinical studies. These include IBI318-bispecific PD-1/PD-L1 antibody (NCT04777084), MGD013-bispecific PD-1/LAG-3 antibody (NCT03219268), RO7247669-bispecific PD-1/LAG-3 antibody (NCT04140500), AZD7789-bispecific PD-1/TIM-3 antibody (NCT04931654), RO7121661-bispecific PD-1/TIM-3 antibody (NCT03708328), AK104-bispecific PD-1/CTLA-4 antibody (NCT05215067), MGD019-bispecific PD-1/CTLA-4 (NCT03761017), AK112-bispecific PD-1/VEGF antibody (NCT04900363), CDX-527 -bispecific PD-L1/CD27 antibody[137] (NCT04440943), GEN1046-bispecific PD-L1/41BB antibody (NCT03917381), INBRX-105-bispecific PD-L1/41BB antibody (NCT03809624), PF-07257876-bispecific CD47/PD-L1 antibody (NCT04881045), GEN1047-bispecific CD3/B7H4 antibody (NCT05180474), XmAb®22841-bispecific CTLA-4/LAG-3 antibody (NCT03849469), and HLX301-bispecific TIGIT/PD-L1 antibody (NCT05102214). Some clinical trials have already shown preliminary results. The bispecific AK104 antibody displayed acceptable safety and four patients showed objective responses among 25 response-evaluable patients.[138] The GEN1046 bispecific antibody targeting PD-L1 and 4-1BB achieved a 65.6% disease-control rate with multiple types of tumors.[139] Although these results are from a phase I study, we anticipate that continuing trials will provide more promising results.

Conclusion

Increasing the survival and treatment response of patients with NSCLC (the most common and deadly cancer worldwide) remains a research hotspot. Although immunotherapy has changed the treatment landscape for NSCLC, some patients do not benefit from current treatments. The TME is considered a crucial determinant of treatment responses.[140] In this review, we discuss the heterogeneity of the TME in NSCLC. Immune cells inside the TME are in a mixed state. Thus, a more accurate portrayal of the immune microenvironment is required. With technological advancements, we should better understand the TME in the future and identify novel targets for drug development. Strategies targeting the immune microenvironment do not focus on the global depletion of all immunosuppressive cells in the TME. The solution should be precisely targeted to distinct cell types using specific antibodies and inhibitors, as mentioned in this review. Bolstering the antitumor phenotype of immune cells may have additional effects. In patients with advanced disease, monotherapy may provide insufficient efficacy. When combined with different drugs (antibodies or inhibitors), chemotherapy or radiation, promising results have been achieved in clinical trials. However, these side effects were non-negligible. Bispecific antibodies have provided new hope. They can simultaneously target two molecules without causing severe side effects. An increasing number of prospective clinical trials are ongoing and have shown promising results. We anticipate that more targeted molecules will be chosen for the development of bispecific antibodies for cancer immunotherapy in the future.

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Conflicts of interest

None.

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