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Nanotechnology: A New Strategy for Lung Cancer Treatment Targeting Pro-Tumor Neutrophils

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ABSTRACT

Primary and metastatic lung cancers are malignant lung tumors each with of which has a different pathogenesis, although both threaten patient lives. Tumor development and progression involve communication between tumor cells and the host microenvironment. Neutrophils are the most abundant immune cells in the tumor microenvironment (TME); they participate in the generation of an inflammatory milieu and influence patient survival through their anti- and pro-tumor abilities. Neutrophils can be classified into various categories according to different criteria; frequent categories include N1 antitumor neutrophils and N2 immunosuppressive neutrophils. The antitumor effects of neutrophils are reported to be mediated through a combination of reactive oxygen species, tumor necrosis factor-related apoptosis-inducing ligand, and receptor for advanced glycation end-products-cathepsin G association, as well as the regulation of the activities of other immune cells. There have also been reports that neutrophils can function as tumor promoters that contribute to lung cancer progression and metastasis by influencing processes including carcinogenesis, angiogenesis, cancer cell proliferation, and invasion ability, as well as having similar roles in the lung metastasis of other cancers. The rapid development of nanotechnology has provided new strategies for cancer treatment targeting neutrophils.

1. Introduction

Of all types of malignant tumors, lung cancer has the highest morbidity and mortality rates, and its occurrence and development are closely related to the lung microenvironment [1–6]. Lung cancer can be classified into two groups, small cell lung cancer (17%) and non-small cell lung cancer (NSCLC; 83%); these groups comprise several subtypes, among which adenocarcinoma is the most common, followed by squamous cell carcinoma (SqCC) and large-cell lung carcinoma, while other types are rare [7–9]. Neutrophils are the most abundant immune cells in the tumor microenvironment (TME) and the earliest immune cells to respond to lung injury and inflammation [10]. Increasing attention has been paid to the relationship between neutrophils and lung cancer development, and many studies have found that neutrophil numbers influence the prognosis of patients with lung cancer [11,12]. Moreover, neutrophil abundance affects patient responses to treatment regimens [13,14]. The underlying mechanisms and factors influencing neutrophils in the context of lung cancer require further exploration.

Neutrophil origin, development, and maturation are complex processes. Previous studies have divided neutrophils into N1 and N2 subtypes, and some researchers have proposed that the proportion of N1 and N2 neutrophils in the TME determines the promotion or inhibition of cancer progression by neutrophils. Neutrophil classification has changed with the development of single-cell sequencing and mass cytometry [15,16], leading to a

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more detailed understanding of neutrophil origin, development, classification, and function.

The development of lung cancer is a continuous and dynamic process that is influenced by neutrophils in various ways. During lung cancer formation, neutrophils can promote tumor cell colonization, survival, and growth [17], dissolve the extracellular matrix (ECM), and increase tumor cell migration and invasion ability. Moreover, neutrophils can promote cancer growth by facilitating micro-vessel formation within tumors [18]. Neutrophil elastase (NE) and neutrophil extracellular traps (NETs) also promote the distant metastasis of lung cancer [19,20]. The cytotoxicity of neutrophils is the most critical mechanism underlying their antitumor effects, and some proteases and cytokines produced by neutrophils also contribute to this process. Similarly, as a type of immune cell, neutrophils can influence the roles of other tumor cells in tumor immunity through various mechanisms [21–23]. The close association of neutrophils with the process of malignant lung tumor occurrence and development has led to a research focus on relevant therapeutic measures targeting neutrophils.

The lung is an organ composed of loose, porous connective tissue with abundant blood flow, and these characteristics make it a target organ for the metastasis of other malignant tumors [24]. Lung metastases of breast cancer, melanoma, and colon cancer have been reported; however, the role of neutrophils in the metastasis process requires further investigation and summary.

In this review, we summarize the impact of neutrophils on patient prognosis and treatment outcomes, update the origin and classification of neutrophils, and discuss the impact of neutrophils on lung cancer development and the promotion of lung metastasis of other cancers by neutrophils. Neutrophils have potential as a new target for lung cancer management and treatment in the future, and the rapid development of nanotechnology has provided new strategies for cancer treatment targeting neutrophils [25–29]. Furthermore, although many treatments targeting neutrophils are currently at a theoretical stage [30–33], their development has been remarkable.

2. Neutrophils in patients with lung cancer

2.1. High pretreatment neutrophil count is associated with low patient survival rate

Over recent decades, numerous studies have focused on the tumor immune microenvironment; almost all immune cells play unique roles in tumor development and metastasis [34–39]. Neutrophils, which are the most abundant immune cells in the body, are a research hotspot. A strong correlation between high neutrophil levels and low survival time in patients with lung cancer has been detected by many researchers [40–45] (Table 1 [11,12,40,43,44,46–53]). To determine the effect of pretreatment peripheral blood neutrophil, lymphocyte, and monocyte counts, as well as the neutrophil-to-lymphocyte ratio (NLR), on patient survival, a randomized controlled trial enrolled 388 chemotherapy (CT)-naïve patients with stage IIIB or IV NSCLC. The study used proportional hazards regression models to estimate hazard ratio (HR) values after adjustment for covariates. The results showed that an elevated pretreatment neutrophil count was associated with shorter overall survival (OS) and shorter progression-free survival (PFS), suggesting that an elevated pretreatment neutrophil count is an independent prognostic factor in patients with advanced lung cancer [54]. Another retrospective study analyzed the gene-expression profiles of tumor tissue samples from 19 NSCLC cohorts, including 18 microarray datasets and one RNA-Seq dataset from The Cancer Genome Atlas lung adenocarcinoma cohort. The percentage of neutrophil infiltration was significantly higher in patients with high recurrence risk than in those with low recurrence risk (5.6% versus (vs) 1.8%), demonstrating that neutrophil infiltration is an independent risk factor in patients with NSCLC [55].

2.2. High neutrophil count is associated with low survival following traditional therapy

In comparison with immunotherapy, which has yet to become fully established, conventional treatments, including surgery, CT, radiotherapy, and targeted therapy (TT), have gained more acceptance. TT and CT are whole-body treatment modalities for lung cancer, similar to immunotherapy, and several clinical trials have shown that high neutrophil counts are strongly associated with poor prognosis of patients undergoing these treatment approaches [46–48] (Table 1). In a brief report, five immune checkpoint inhibitor (ICI) trials and six TT trials, comprising 1368 patients treated with ICIs and 1072 patients treated with CTs in ICIs trials (total n = 2440), were analyzed. The researchers used the derived neutrophil-to-lymphocyte ratio (dNLR), calculated as absolute neutrophil count/(white blood cell count – absolute neutrophil count), as an evaluation index. High dNLR values were associated with shorter OS (HR = 1.64, $p < 0.001$) and PFS (HR = 1.26, $p = 0.008$) in patients treated with ICIs and were an independent prognostic factor. For patients treated with CT, dNLR ≤ 3 was associated with a longer survival time (HR = 0.49, $p < 0.001$). Multivariate analysis showed that albumin level, lactate dehydrogenase (LDH) level, and dNLR could serve as independent prognostic factors. In an analysis of six TT trials, patients with low dNLR had a median survival of 46.5 months, compared with 16.6 months for those with high dNLR treated with TTs (HR = 0.28). Furthermore, among patients receiving CT, those with a low dNLR value had more prolonged survival than those with high dNLR (estimated median survival, 33.4 vs 17.1 months; HR = 0.41), while high dNLR and LDH levels were independently associated with poor OS (TTs, $p < 0.001$; CTs, $p < 0.001$) and PFS (TTs, $p < 0.001$; CTs, $p < 0.001$) in a multivariate analysis [49]. A total of 182 patients with NSCLC treated with CT were enrolled in another study, where the NLR was defined as the absolute neutrophil count divided by the absolute lymphocyte count. The resulting data indicated that low pretreatment NLR (≤ 2.63) (odds ratio (OR) = 2.043, $p = 0.043$) and decreased post-treatment NLR (OR = 2.368, $p = 0.013$) were associated with the response to CT. Pretreatment NLR was also an independent prognostic factor for PFS (HR = 1.807, $p = 0.018$) and OS (HR = 1.761, $p = 0.020$) [50]. Patients needing CT or TT typically have stage III or IV disease. Hence, these results may be due to either advanced cancer, in which the baseline neutrophil count is high, or neutrophil-mediated suppression of T lymphocytes. Therefore, data from large patient cohorts—particularly those with early-stage lung cancer—are required.

Surgery and radiotherapy are local treatment methods for malignant tumors and are the first choice for early- and mid-stage lung cancer therapy. Similar conclusions have been obtained for surgery and radiotherapy. A retrospective study on 485 surgically resected patients with solitary lung SqCC found that high levels of tumor-infiltrating $CD10^{+}$ neutrophils were associated with worse patient prognosis ($p = 0.021$) (Table 1). The researchers also constructed tissue microarrays and performed immunostaining for cluster of differentation 3 (CD3), protein tyrosine phosphatase receptor type C, CD8, CD4, forkhead box P3 (FoxP3), CD20, CD68, C–X–C motif chemokine ligand 12 (CXCL12), C–X–C motif chemokine receptor 4 (CXCR4), C–C motif chemokine receptor 7 (CCR7), interleukin-7 (IL-7) receptor, and IL-12. The results of an analysis of biologically relevant immune cell combinations showed that patients with $CD10^{high}$ neutrophils and $CD20^{low}$ lymphocytes had significantly worse OS (5-year OS, 42%) than those

Table 1
Landmark studies evaluating the prognostic role of neutrophils in lung cancer clinical trials.

Year	Tumor type	Number	Parameter analyzed	Cut-offs	Clinical endpoint	Clinical outcome	Therapy	Study design	Significant in multivariate analysis
2020 [53]	NSCLC	50	NLR	> 3	OS	Worse	ICIs	Prospective	HR = 1.191, p = 0.01
2020 [47]	NSCLC	751	NLR	> 3.5	OS	Worse	ICIs	Prospective	p < 0.001
					PFS	Worse			p < 0.05
2018 [48]	NSCLC	134	ANC	> 7500 μL^{-1}	OS	Worse	ICIs	Retrospective	HR = 3.97, p = 0.03
					PFS	Worse			HR = 3.97, p = 0.001
2017 [11]	NSCLC	52	NLR	> 5	OS	Worse	ICIs	Prospective	HR = 5.01, p < 0.001
					PFS	Worse			HR = 2.09, p = 0.007
2017 [51]	NSCLC	59	NLR	> 3.44	OS	Worse	Radiotherapy	Retrospective	HR = 1.25, p = 0.012
2019 [52]	NSCLC	335	dNLR	> 4	OS	Worse	ICIs	Retrospective	HR = 2.30, p = 0.008
2019 [49]	mNSCLC	1368	dNLR	> 3	OS	Worse	ICIs	Retrospective	HR = 1.64, p < 0.001
					PFS	Worse			HR = 1.26, p = 0.008
		1110	dNLR	> 3	OS	Worse	TT	Retrospective	HR = 1.87, p < 0.001
					PFS	Worse			HR = 1.54, p < 0.001
2018 [12]	NSCLC	466	dNLR	> 3	OS	Worse	ICIs	Retrospective	HR = 2.22, p = 0.008
					PFS	Worse			HR = 1.83, p = 0.015
2015 [43]	LUSC	485	NLR	> 5.5	OS	Worse	Surgery	Retrospective	p = 0.021
2015 [44]	ES-SCLC	555	NLR	> 4.4	OS	Worse		Retrospective	p < 0.001
	LS-SCLC	383	NLR	> 3.1	OS	Worse			p < 0.001
2014 [40]	SCLC		NLR	> 4	OS	Worse	CT	Retrospective	p = 0.019
					PFS	Worse			p = 0.005
2013 [50]	NSCLC	182	NLR	> 2.63	OS	Worse	CT	Prospective	HR = 1.761, p = 0.020
					PFS	Worse			HR = 1.807, p = 0.018
2015 [46]	NSCLC	149	NLR	> 2.98	OS	Worse	Radio	Retrospective	p = 0.005

ICI: immune checkpoint inhibitor; ANC: absolute neutrophil count; dNLR: derived neutrophil-to-lymphocyte ratio; NLR: neutrophil-to-lymphocyte ratio; mNSCLC: metastatic NSCLC; CT: chemotherapy; TT: targeted therapy; LUSC: lung squamous cell carcinoma; SCLC: small cell lung cancer; ES-SCLC: extensive stage SCLC; LS-SCLC: limited stage SCLC; OS: overall survival; PFS: progression-free survival; HR: hazard ratio.

with other CD10 and CD20 combinations (5-year OS, 62%; p < 0.001). Furthermore, multivariate analysis demonstrated that the $CD10^{high}/CD20^{low}$ immune cell combination was an independent predictor of OS in both the training (HR = 1.61, p = 0.006) and validation (HR = 1.75, p = 0.043) cohorts [43].

In the field of radiotherapy, a report on 59 patients with NSCLC treated with salvage stereotactic ablative radiotherapy (SABR) found that a high NLR after SABR predicted a low survival rate (HR = 1.25, p = 0.012) [51]. Due to the specific role of neutrophils in tumors, another retrospective study on patients undergoing SABR found that an appropriate NLR cutoff value for survival analysis was 2.98, based on receiver operating characteristic (ROC) curve analysis. Similarly, neutrophils are associated with genetic mutations in NSCLC [52,56]. Although neutrophils have been shown to have antitumor effects in many experiments, in addition to promoting tumor progression, few clinical trials have provided evidence to conclusively support the antitumor influence of neutrophils.

2.3. High neutrophil count is associated with low survival following immunotherapy

Immunotherapy, which aims to inhibit tumor progression by improving the immune microenvironment in patients with cancer, has emerged as a new direction in the last decade [38,39]. Numerous studies have shown the importance of neutrophil levels in determining the effectiveness of various treatment methods, including ICIs, an indispensable type of immunotherapy [46–48,57] (Table 1). Eleven randomized clinical trials were analyzed, including 4614 patients with metastatic NSCLC (mNSCLC). To strengthen the prognostic power of dNLR, a surrogate for NLR in NSCLC, lung immune prognostic index (LIPI) composite scores, calculated based on the dNLR (absolute neutrophil count/(white blood cell count – absolute neutrophil count)) and LDH level, were used as a key evaluation indicator in patients with mNSCLC who received ICIs—more specifically, programmed death-1 (PD-1)/programmed death-ligand 1 (PD-L1) inhibitors. The LIPI includes dNLR > 3 and LDH levels above the upper limit of normal (ULN) and divides patients into three risk groups: good, intermediate, and poor. A good LIPI score (LDH level < ULN and dNLR $\leq$ 3) was associated with longer OS compared with a poor LIPI score in the ICI-treated group, with estimated median survival times of 15.6 vs 4.5 months (HR = 0.34, 95% confidence interval (95% CI): 0.28–0.42). A similar prognostic association was observed in patients receiving CT, where those with good LIPI scores survived longer than those with poor scores (estimated median survival, 10.4 vs 5.3 months; HR = 0.49, 95% CI: 0.40–0.60). Furthermore, multivariate analysis showed that albumin levels, LDH levels < ULN, and dNLR $\leq$ 3 were all associated with longer OS and PFS [49]. In another multicenter retrospective study, researchers found that pretreatment dNLR and LDH levels were correlated with ICI treatment outcomes in patients with NSCLC. The study was conducted in eight European centers and divided patients into three groups, according to therapy methods, as follows: test (n = 161) and validation (n = 305) groups treated with PD-1/PD-L1 inhibitors; and a control cohort (n = 162), treated with CT only. Complete blood counts and LDH and albumin levels were measured prior to ICI treatment, and a LIPI was developed based on dNLR > 3 and LDH > ULN and was used to classify patients into three groups (good, intermediate, and poor). In the pooled ICI treatment cohort (n = 466), dNLR > 3 and LDH > ULN were independently associated with OS (p < 0.001, HR = 2.22, 95% CI: 1.23–4.01 and p < 0.001, HR = 2.51, 95% CI: 1.32–4.76, respectively). Median OS times for patients with poor, intermediate, and good LIPI were 3 (95% CI: 1 to not reaching (NR)), 10 (95% CI: 8 to NR), and 34 (95% CI: 17 to NR), respectively, and median PFS times were 2.0 (95% CI: 1.7–4.0), 3.7 (95% CI: 3.0–4.8), and 6.3 (95% CI: 5.0–8.0) months (all p < 0.001). However, no significant differences in survival were detected in the CT cohort [12].

In another clinical study involving nivolumab (an anti-programmed death receptor-1 antibody), including 52 patients with mNSCLC [11], a Cox regression analysis was used to investigate the prognostic value of NLR for OS and PFS. The study found that a high NLR was associated with poor OS (HR = 3.64, 95% CI:

1.78–7.46, $p < 0.001$). The researchers concluded that NLR was only an independent prognostic factor for mNSCLC treated with nivolumab.

In a prospective clinical study, data—including complete blood count at baseline within one week prior to the first nivolumab infusion—from eligible patients with metastatic or non-resectable NSCLC who could not receive curative treatment were analyzed retrospectively. Patients with co-infections, including human immunodeficiency virus or hepatitis, receiving systemic steroids or combined radiotherapy, or with previous or ongoing autoimmune disease, were excluded. Median OS and PFS were 9.6 (95% CI: 6.0 – > 14.0) and 2.1 (95% CI: 1.8–6.4) months, respectively, in the entire group of patients included in the analysis. Higher NLR was associated with trends toward shorter OS and PFS; however, significant differences in OS were detected among patients classified into three groups based on NLR quartiles. A higher than median NLR was also associated with worse OS (NR vs 5.1 months; HR = 3.3, 95% CI: 1.3–8.5, $p = 0.013$ and 4.8 vs 13.2 months; HR = 4.1, 95% CI: 1.5–11.3, $p = 0.006$, respectively). Correspondingly, the relationship between NLR and response to nivolumab treatment was significant in a model that included NLR and first-line response as predictors ($p = 0.028$) [53].

In summary, almost all published findings suggest that NLR or dNLR may be valuable tools for predicting the efficacy of immunotherapy or TT and informing decisions on whether more effective combination therapies should be used for patients with lung cancer. Hence, treatment effects may be improved by reducing patient neutrophil count before and during the ICI treatment. Therefore, investigation of whether the regulation of neutrophils can influence the effect of ICI treatment is warranted to develop new types of immunotherapy that combine ICI treatment with neutrophil-targeting agents, with the potential to provide better outcomes for patients with lung cancer.

3. Classification of neutrophils in lung cancer

3.1. Neutrophil origin and differentiation in lung cancer

Neutrophils differentiate in the bone marrow from common myeloid progenitor cells to typical granulocyte–monocyte progenitor cells; this process is followed by several further stages, including myeloblasts, promyelocytes, myelocytes, metamyelocytes, and mature neutrophils [58–60]. Neutrophils are short-lived white blood cells of the innate immune system that require constant replenishment by newly differentiated cells from the bone marrow. During this process, various granules are formed, which store different components including primary azurophilic granules, secondary specific granules, tertiary gelatinase granules, and secretory vesicles [59,61]. The differential release of various granules from neutrophils contributes to the heterogeneity, multifunctionality, and plasticity of the neutrophils observed in various pathophysiological conditions. Exposure to different combinations of cytokines and chemokines regulates the activation state mature, normal, and urgent neutrophils. Depending on the stimuli and stage of differentiation, neutrophil contents are discharged into the surrounding environment via cytosolic action, while their membrane components are expressed on the cell surface [62–64], allowing neutrophils to respond rapidly to stimuli in their surrounding environment.

Numerous clinical studies have found that neutrophils can have pro-tumor-growth and metastasis effects and have demonstrated neutrophils' pro-metastatic roles by eliminating neutrophils in animal cancer models, leading to decreased metastatic seeding [65–68] (Table 2 [65–80]). Nevertheless, other studies have reported that the similar elimination of neutrophils in some animal models leads to increased metastasis, suggesting that neutrophils can also have anti-metastatic effects [81–83] (Table 3 [81–95]). When neutrophil depletion is performed using the same antibody, it can result in tumor progression and/or tumor suppression outcomes (Tables 2 and 3). The effects of neutrophil depletion in opposing tumor growth and metastasis can be partly explained by the differential activation of neutrophils by tumor cells and other cells in the tumor environment, which results in different ratios of pro-tumor and antitumor neutrophils [96,97]. Hence, neutrophils require more precise classification according to their specific structures and functions.

Neutrophils can be divided into different groups according to various classification methods. Neutrophils are commonly classified according to their cell surface markers. For example, neutrophils with the cell surface markers $CD15^+$, $CD16^+$, $CD66b^+$, and $CD14^-$ are categorized as human neutrophils, while neutrophils with the markers $CD11b^+$, $Ly6G^+$, and $Ly6C^-$ are mouse neutrophils [98]. The names of neutrophils vary widely, depending on these different classification methods, particularly in malignant tumors. The most commonly reported neutrophil populations closely associated with lung cancer include N1/N2 neutrophils, tumor-associated neutrophils (TANs), low-density neutrophils (LDNs), and polymorphonuclear myeloid-derived suppressor cells (PMN-MDSCs). During tumor progression, granulocyte colony-stimulating factor (G-CSF) and granulocyte–macrophage colony-stimulating factor (GM-CSF) are secreted into the blood circulation by cancer tissues and stimulate bone marrow hematopoietic cells to secrete LDNs, high-density neutrophils (HDNs), PMN-MDSCs, monocytic myeloid-derived suppressor cells (M-MDSCs), and other types of neutrophil. All of these neutrophil types have remarkably similar morphological features and surface biological markers and can transform into other types in response to tumor-secreted cytokines, making the study of the evolution of neutrophils and their functional mechanisms in tumor progression challenging (Table 4 [16,99–124] and Table 5 [114–116,118–120]).

Regardless of the cell type, LDN, HDN, PMN-MDSC, M-MDSC, or N1 and N2 TANs, definitions of cell populations are based on cell origin and the specific acquisition method. There is no complete cytological definition of neutrophil surface and biological markers. Furthermore, cell types can intersect; for example, immunosuppressive LDNs identified in the peripheral blood of patients with cancer have a phenotype consistent with that of PMN-MDSCs [98,125,126]. CD10 (also known as common acute lymphoblastic leukemia antigen, neutrophil endopeptidase, or enkephalinase) can be used as a marker to clearly distinguish between mature $CD66b^+CD10^+$ and immature $CD66b^+CD10^-$ neutrophils in heterogeneous populations of circulating $CD66b^+$ neutrophils that emerge under inflammatory conditions [127]. $CD66b^+CD10^+$ neutrophils inhibit T cell proliferation and interferon-γ (IFN-γ) production through the CD18-mediated release of contact arginase 1 (Arg1). In contrast, $CD66b^+CD10^-$ neutrophils can manifest the opposite behavior, promoting T cell survival, proliferation, and IFN-γ production via CD18-mediated contact-dependent mechanisms. Given this complexity, we surveyed cell surface and biological markers for several species of neutrophils commonly found in tumors (Table 4). Unfortunately, we did not identify a clear basis for neutrophil delineation, which may require more studies of subcellular populations.

Temporal physiological processes are major sources of neutrophil heterogeneity; numerous studies have found that tumor-associated aged neutrophils (Nageds; $CXCR4^+CD62L^{low}$) have a robust pro-metastatic capacity [128–131]. Nageds ($CXCR4^+CD62L^{low}$ neutrophils) can be obtained by downregulating CD62L and upregulating CXCR4 in $CXCR4^{low}CD62L^{high}$ naïve neutrophils [128]. They promote tumor migration and support tumor metastasis by increasing the release of several pro-metastatic factors, including

Table 2
Example of tumor models showing pro-tumor neutrophil function.

Tumor model	Neutrophil characteristics	Pathways of pro-tumor progression
Chronic wound inflammation-induced melanoma [65]	$CD66b^+$ neutrophils	Delaying the development of neutrophils using morpholinos to G-CSF reduced the number of premetastatic cells. The density of $CD66b^+$ neutrophil infiltration is an independent prognostic marker of poor melanoma-specific survival
Kras-driven mouse model of lung cancer [75]	$Gr1^+$ neutrophils	Depletion experiments showed that Gr1 cells favor tumor growth, reduce T cell homing, prevent successful anti-PD1 immunotherapy, and alter angiogenesis
A model of invasive intestinal adenocarcinoma [76]	$Ly6G^+$ neutrophils	Ly6G cell depletion purged CXCR2-dependent tumor-associated leukocytes and suppressed established skin tumor growth and colitis-associated tumorigenesis
MMTV-PyVT spontaneous breast cancer model [66]	$Ly6G^+$ neutrophils	GM-CSF levels were increased in collagen-dense tumors. Depleting neutrophils with anti-Ly6G reduced tumor burden and the number of lung metastases
Metastasis models by injecting 4 T1 and E0771 cells [72]	$Ly6G^+$ neutrophils	Significant inhibition was observed in neutrophil-depleted mice by injecting the anti-Ly6G (clone 1A8) monoclonal antibody
Breast cancer cells MDA-MB-468 [67]	$Ly6G^+$ neutrophils	Depletion of neutrophils decreased the metastatic spread of polyclonal tumors driven by IL-11 and FIGF subclones. Induction IL-11/FIGF expression upregulated the expression of chemoattractants for pro-metastatic neutrophils, including CXCL12, CXCL14, and CXCL1
66c14 breast carcinoma cells [77]	$Ly6G^+Ly6C^+$ neutrophils	G-CSF-mobilized $Ly6G^+Ly6C^+$ cells were created to produce the Bv8 protein, which stimulated tumor cell migration by activating one of the Bv8 receptors—prokineticin receptor-1. Anti-G-CSF or anti-Bv8 antibodies significantly reduced lung metastasis
B16F10 melanoma or MCA205 fibrosarcoma cells [69]	$CD11b^+Gr1^+$ neutrophils	These tumors showed an enhanced infiltration of $CD11b^+Gr1^+$ neutrophils expressing elevated levels of genes encoding VEGF, MMP9, CXCR4, and the transcription factors c-myc and STAT3. Treatment with low levels of IFN-β inhibited tumor growth
Breast cancer cells including MDA-MB-231, MCF10CA1h, and 4 T1 [73]	$CD11b^+Ly6G^+$ neutrophils	CTSCs promoted the lung colonization of breast cancer by both recruiting and activating neutrophils. The CTSC–PR3–IL-1β axis of the neutrophils contributed to mitogen-activated protein kinase 14 activation and ROS production, leading to NET formation to support the metastatic growth of tumor cells
Lung metastatic MMTV-PyMT mammary tumor mouse model [71]	$CD11b^+Ly6G^+$ neutrophils	Depletion of neutrophils harboring primary tumors during the premetastatic stage led to decreased metastatic seeding. Neutrophil Alox5 inhibition may limit metastatic progression
HT29, HCT-116, LoVo, Pan02, and KPC cells [78]	$CD11b^{high}/Gr1^{high}$ neutrophils cells	Depletion of neutrophils in established experimental murine liver metastases led to diminished metastatic growth. CXCR2-expressing neutrophils contribute to angiogenesis through the secretion of FGF2
Chemically induced cutaneous SqCC [68]	$Gr1^{bright}/Ly6G^+$ neutrophils	Depletion of neutrophils delayed tumor growth and increased the frequency of proliferating IFN-γ-producing $CD8^+$ T cells. Mechanisms that limited antitumor responses involved high arginase activity, the production of ROS and NO, and the expression of PD-L1 on TAN, concomitantly with the induction of PD-1 on $CD8^+$ T cells
LPS-induced lung inflammation model for metastatic seeding of B16-BL6 melanoma and LLC Lewis lung carcinoma cells [74]	$CD45^+CD11b^+Ly6G^+$ neutrophils	Neutrophils expressed the inflammatory mediators IL-1β, TNF-α, IL-6, and COX2. The NE and cathepsin G degraded Thrombospondin 1, facilitating metastatic seeding in the lung. Depletion of neutrophils suppressed LLC lung metastases
A spontaneous breast cancer model (K14cre, Cdh1F/F, Trp53F/F, KEP) mice [79]	Neutrophils, defined as $CD45^+CD11b^+Ly6G^+Ly6C^+F4/80^-$ cells	IL-1β elicited IL-17 expression from gamma delta (γδ) T cells, resulting in G-CSF-dependent expansion and the polarization of neutrophils. Tumor-induced neutrophils acquired the ability to suppress cytotoxic T lymphocytes carrying the CD8 antigen
The 4 T1 breast cancer cell line [80]	$Gr1^+$ cells; $Ly6G^+$ cells; $CD11b^+/Ly6G^+/MMP9^+$ neutrophils	$Ly6G^+$ neutrophils play an important positive role in colonizing and growing breast cancer cell metastasis to the liver. The expression of molecules that have been reported as being preferentially expressed in N2-polarized neutrophils—such as MMP9, CCL5, or arginase 1—increased during liver metastasis progression
Lewis lung carcinoma H-59 cells [70]	PMN isolated from C57BL6 mice	Using the anti-GR1 antibody clone RB6-8C5, mice depleted of circulating neutrophils reduced gross metastatic foci from a median of 45 in control mice to 4. This effect appears to be almost entirely mediated by Mac-1

G-CSF: granulocyte colony-stimulating factor; GM-CSF: granulocyte–macrophage colony-stimulating factor; VEGF: vascular endothelial growth factor; CTSC: tumor-secreted protease cathepsin C; ROS: reactive oxygen species; PyMT: polyoma middle T antigen; Alox5: arachidonate 5-lipoxygenase; TAN: tumor-associated neutrophil; CCL5: C—C motif chemokine ligand 5; TNF-α: tumor necrosis factor-α; PMN: polymorphonuclear; MMP9: matrix metalloproteinase 9; PR3: proteinase 3; MMTV-PyVT: the mouse mammary tumor virus-the Polyoma Virus middle T antigen; Ly6G: lymphocyte antigen 6complex,locus g; CD: cluster of differentiation; Gr1: myeloid differentiation antigen; FIGF: free insulin-like growth factor; Bv8: prokineticin 2; c-myc: MYC proto-oncogene; bHLH: transcription factor; STAT3: signal transducer and activator of transcription 3; IFN-β: interferon-β; FGF2: fibroblast growth factor 2; NO: nitrogen monoxide; LPS: lipopolysaccharide; LLC: Lewis lung carcinoma; COX2: cytochrome c oxidase subunit II; Mac-1: macrophage associated antigen-1.

NETs, reactive oxygen species (ROS), vascular endothelial growth factor (VEGF), and matrix metalloproteinase 9 (MMP9). Another study focused on the presence and biological function of Nageds and found that they can generate sirtuin 1 (SIRT1)-induced NETs dominated by mitochondrial DNA [129]. Based on these results, various interventions centered on SIRT1–Nageds have been conducted; for example, vitamin B3 inhibits the SIRT1 activation of neutrophils by tumor cells, Mdivi-1 blocks mitosis and promotes Naged death, TRO19622 blocks DNA release from mitochondrialpermeability transition pore (mPTP) channels, and deoxyribonuclease I (DNase I) clears NETs. These drugs can prevent tumor metastasis to varying degrees but do not have noticeable

Table 3
Example of tumor models showing antitumor neutrophil function.

Tumor model	Neutrophil characteristics	Pathways of antitumor progression
RM1 prostate cancer model [86]	PMNs	Neutrophil depletion prevented the rejection of tumor cells induced by adenovirus-mediated IL-12 gene therapy
SBcl2 primary melanoma cells [87]	$Ly6G^+$ neutrophils	Depletion of neutrophils enabled the growth and survival of IL-8-overexpressing melanoma cells
4 T1 breast cancer cells [82]	$Ly6G^+$ neutrophils	Elimination of neutrophils resulted in increased lung metastases. TENs inhibited metastatic lung seeding by generating H_2O_2
CXCL5-overexpressing B16F1 melanoma [81]	$Ly6G^+$ neutrophils	Neutrophil depletion in *CXCL5*-overexpressing tumor-bearing mice caused increased metastasis formation
SN12C or LM2 renal carcinoma [83]	$Ly6G^+$ neutrophils	Depletion of neutrophils caused an increased rate of metastatic colonization. IL-8 could activate the antitumor neutrophil function
E0771 breast cancer cells [88]	$Ly6G^+$ neutrophils	In NK cell-deficient mice, granulocyte colony-stimulating factor-expanded neutrophils showed an inhibitory effect on the metastatic colonization of breast tumor cells in the lung
4 T1, LLC, 4 T07, and 168FARN cells [85]	$Ly6G^+$ neutrophils	Neutrophil depletion dramatically enhanced the seeding of control tumor cells in the lungs, confirming the protective role neutrophils play in the premetastatic lung.
Siglec-9-ligand-positive carcinoma cells [89]	$Ly6G^+$ neutrophils	The sialic acid-dependent binding of tumor-associated ligands to Siglec-9 and -E inhibited neutrophils and increased lung colonization in an experimental metastasis assay
C57Bl/6 syngeneic B16 F10 melanoma and Lewis lung carcinoma models [90]	$Ly6G^+$ neutrophils	Genetic deletion of *Ppm1d* in immune cells suppressed the growth of isograft tumors in immune-competent mice through increased infiltration of neutrophils
Lewis lung carcinoma [91]	$CD11b^+Ly6G^+$ neutrophils	Neutrophil depletion augmented tumor growth rate, increased the percentages of IL-17-producing cells, and reduced the percentages of $CD8^+$ T cells in tumors
TGF-β blockage of AB12 mesothelioma cells [84]	$CD11b^+Ly6G^+$ neutrophils	Depletion of neutrophils significantly blunted the antitumor effects of treatment and reduced $CD8^+$ T cell activation
Breast cancer [92]	$CD11b^+Gr1^+$ neutrophils	Neutrophils became TMEM173 in a lung microenvironment infiltrated with CCR2 monocytes surrounding apoptotic metastatic breast cancer cells, supporting myeloid cooperation against metastatic breast cancer
4 T1 mouse mammary carcinoma [93]	$CD11b^+Ly6G^{high}Ly6C^{low}$ neutrophils	RLI treatment restored the balance between NK cells and neutrophils ($CD11bLy6G^{high}Ly6C^{low}$) that massively infiltrated the lungs of 4 T1 tumor-bearing mice
Lung cancer [94]	$CD11b^+CD15^{hi}CD66b^+MPO^+Arg1^+CD16^{int}IL\text{-}5R\alpha^-$ cells uman ($CD11b^+$, $CD14^+$, $CD15^+$,	Hybrid neutrophils triggered and significantly augmented the activation of antigen-specific effector T cells
Prostate cancer cells (C42B, PAIII, and LNCaP) [95]	$CD16^+$, $CD10^+$), mouse ($CD45^+$, $CD11b^+$, $Ly6G^+$)	Neutrophil depletion in bone metastasis models enhanced BM-PCa growth. Neutrophil-mediated PCa killing was found to be mediated by the suppression of STAT5

TEN: tumor-entrained neutrophil; NK: natural killer; Ppm1d: protein phosphatase, Mg^{2+}/Mn^{2+} dependent 1D; TGF-β: transforming growth factor-β; TMEM: recombinant transmembrane protein; RLI: imbruvica; BM: bone metastatic; PCa: prostate cancer; Arg1: arginase 1; MPO: myeloperoxidase.

effects on primary tumors, indicating that methods to deliver drugs directly to tumors are urgently required.

3.2. Polymorphonuclear myeloid-derived suppressor cells(MDSCs)

MDSCs are heterogeneous cell populations derived from bone marrow progenitors and immature myeloid cells. To date, two significant subpopulations of MDSCs have been identified: M-MDSCs and PMN-MDSCs [117,121,132]. PMN-MDSCs share phenotypic and morphological features with neutrophils, whereas M-MDSCs are similar to monocytes. Mouse MDSCs have a $CD11b^+Gr1^+$ phenotype and are divided into two subtypes: $CD11b^+Ly6G^+Ly6C^-$ granulocytic MDSCs (also referred to as PMN-MDSCs) and $CD11b^+Ly6G^-Ly6C^+$ M-MDSCs [133]. Human MDSCs are defined as M-MDSCs (human leukocyte antigen (HLA)-$DR^-CD11b^+CD14^+CD15^-CD33^+CD66b^-$) and PMN-MDSCs ($HLA\text{-}DR^-CD11b^+CD14^-CD15^+CD33^+CD66b^+$). MDSCs exert immunosuppressive functions, mainly through the production of Arg1, nitric oxide synthase 2 (NOS2), and ROS [122]. PMN-MDSCs, which are an essential subpopulation of MDSCs mainly found in peripheral blood, were initially identified in mouse tumor models and have immunosuppressive effects that assist tumor progression (Fig. 1). Relative to neutrophils, PMN-MDSCs show similar surface marker expression but lack specific surface antigens, and there is now a consensus that they should have at least the surface markers $CD66b^+$, $CD16^+$, $CD15^+$, $CD11b^+$, and $CD33b^{mild}$, and be HLA-DR-negative [134–136]. Traditionally, PMN-MDSCs can be divided into subgroups: $CD11b^+Ly6G^+$ and $Ly6C^-$ [137,138]. Although PMN-MDSCs are primarily considered to foster cancer development, despite wielding an arsenal of cytotoxic agents, a surprising inhibitory role played by PMN-MDSCs in epithelial carcinogenesis was described based on experiments using a mouse model of phosphatase and tensin homolog (PTEN)-deficient uterine cancer [139]. By inducing tumor cells to detach from the basement membrane, PMN-MDSCs impede early tumor growth and inhibit malignant tumor progression. Unexpectedly, PMN-MDSC recruitment and tumor growth control occur independently of lymphocyte and cellular senescence but are part of the innate inflammatory response of tumors to hypoxia. In another study, three populations of PMNs were detected in tumor-bearing mice: classical PMNs, PMN-MDSCs, and activated PMN-MDSCs (aPMN-MDSCs), where aPMN-MDSCs have potent immunosuppressive activity and are only found in tumors.

PMN populations in patients with cancer and in tumor-bearing mice have striking similarities. Two distinct PMN populations found in tumor-bearing mice, with the characteristics of classic PMNs and PMN-MDSCs, are also present in the peripheral blood of patients with cancer. In contrast, the genetic profiles of PMN-MDSCs in human tumors are similar to those of aPMN-MDSCs in mice and are associated with unfavorable clinical

Table 4
Selected surface markers and bio-makers of TANs, LDNs, HDNs, M-MDSCs, and PMN-MDSCs in humans.

Marker	TANs [16,99–102]		LDNs [103–108]	HDNs [109–113]	MDSC [114–124]	
	N1	N2			M-MDSC	PMN-MDSC
CD10	–	+++	+/–	+/–		
CD11b	+++	++	+++	++	++	+++
CD13			+++	++	+	+++
CD14			+	++	++	–
CD15	+++	++	+++	++	–	+++
CD16 (IGFR3)	+++	++	+	+++	++	+/–
CD32	+++				+	+
CD33	+++	++	+++	++	+++	+
CD34			–		+++	+
CD35			++	+	–	–
CD36			+++	–		
CD38					++	++
CD39					++	++
CD41			+++	–		
CD45			+++	+/–	++	++
CD54 (ICAM-1)	++	+/–			+++	+
CD61			+++	–		
CD62L	+++	++	+	++	++	++
CD63			+++	++		
CD64			+++	++		
CD66b	+++	++	+++	++	–	+++++
CD68					++	–
CD73					++	++
CD80					+/–	–
CD83					+/–	–
CD86			–	+	+/–	–
CD107a			++	+/–		
CD115					+	+/–
CD117					+/–	++
CD119 (IFNGR)	+	+++				
CD124					++	++
CD163					++	–
CD178 (FASLG)	+/–	+++				
CD181 (CXCR1)	++	+++			++	++
CD182 (CXCR2)	++	+++		++	++	++
CD184 (CXCR4)	+	+++	+++	++	++	++
CD192 (CCR2)					+++	++
CD226			+++	–		
CD253 (TRAIL)	+/–	+++				
CD274 (PDL1)			++	+/–	+/–	++
CD309 (VEGFR)	+	+++	+++	++	+	+
LOX1			++	–	–/+	–/+
HLA-DR	–	–	++	–	–	–
ROS	++	+++	+	+++	++	+++
ARG1	+	+++	++	++	+++	++
MPO	++	++			++	+++
NOS2	+++	++			+++	++
MMP9	+	+++				
S100A9			+++	++	+	++
STAT3	+	+++			+/–	–
TNF-α	+	+/–				
IFN-β	+	++				
ADGRG5	–	+++				
CCL2	+/–	++				
CCL3	++	+++				
CCL4	++	+++				
CCL5	+	+++				
CCL8	++	+++				
CCL12	++	+++				
CCL17	–	+++				
CXCL1	++	+++				
CXCL2	+	++				
CXCL6						
CXCL10	+/–	+				
CXCL11	+	+				

PMN: polymorphonuclear; ICAM-1: intercellular adhesion molecule-1; TRAIL: tumor necrosis factor-related apoptosis-inducing ligand; MDSC: myeloid-derived suppressor cell; IGFR3: insulin like growth factor 1 receptor 3; IFNGR: interferon-γ receptor; FASLG: Fas ligand; PDL1: programmed cell death ligand 1; VEGFR: vascular endothelial growth factor receptor; LOX1: lipoxygenase 1; HLA-DR: human leukocyte antigen DR; ARG1: arginase 1; S100A9: S100 calcium binding protein A9; ADGRG5: adhesion G protein-coupled receptor G5; NOS2: nitric oxide synthase 2..

Table 5
Selected surface markers and bio-markers of M-MDSCs and PMN-MDSCs in mice.

Marker	MDSCs [114–116,118–120]	
	M-MDSCs	PMN-MDSCs
CD1b	++	–
CD1d	+	–
CD2 (LFA-2)	+	–
CD11a (LFA-1)	++	++
CD11b (Mac-1)	++	++
CD13	–	–
CD14	–	–
CD16 (Fcgr3)	+	+
CD21 (Cr2)	–	–
CD23	–	–
CD31 (PECAM-1)	+	–
CD32 (Fcgr2)	+	+
CD34	–	–
CD35 (Cr1)	–	–
CD38	++	++
CD39	++	++
CD40	–	–
CD43	+++	+++
CD44	+++	+++
CD45	++	++
CD49DB	+++	+
CD54	+++	+
CD62L (L-selectin)	+++	+++
CD71	+	–
CD73	++	+++
CD80 (B71)	++	++
CD86 (B72)	–	–
CD98	+	++
CD115 (M-CSFR)	+	+/–
CD117 (c-KIT)	–	–
CD120b (TNF-R2)	+	+
CD124 (Il4ra)	+/–	+/–
CD162 (Psgl-1)	+++	+++
CD192 (Ccr2)	++	+
CD195 (Ccr5)	++	++
CD274 (PD-L1)	+	+/–
CD181 (Cxcr1)	++	++
CD182 (Cxcr2)	++	++
CD184 (Cxcr4)	++	++
CD204 (MRS-A)	–	–
CD209	–	–
CD273 (PD-L2)	+/–	+/–
CD301a/b	+	–
CD309 (VEGFR)	++	++
Gr1	+	+++
Ly6A	++	++
Ly6E	+/–	–
Ly6C	+++	+/–
Ly6G	–	+++
F4/80	++	+/–
Cx3cr1	–	–
Ros	+	+++
Nos2	+++	+
Arg1	++	++
Mac-2 (Galectin-3)	++	+
MHC Class I	+++	+++
MHC Class II	–	–

LFA: lymphocyte function-associated antigen; Mac: macrophage galactose-specific lectin; Fcgr: Fc gamma receptor; Gr: glutathione reductase; PECAM: platelet/endothelial cell adhesion molecule; M-CSFR: colony stimulating factor; c-KIT: KIT proto-oncogene receptor tyrosine kinase; Il4ra: interleukin 4 receptor α; Psgl: p-selectin ligand; MRS-A: macrophage scavenger receptor 1; MHC: major histocompatibility complex; F4/80: adhesion G protein-coupled receptor E1; Cx3cr1: C–X3–C motif chemokine receptor 1; Ros: ROS proto-oncogene 1, receptor tyrosine kinase.

outcomes in patients with cancer. These findings demonstrate that aPMN-MDSCs may be a unique PMN population with tumor immunosuppressive functions. Furthermore, single-cell sequencing of $CD45^{+}Ly6G^{+}Ly6C^{low}$ PMN cell populations from tumor-bearing mice showed that aPMN-MDSCs can be classified according to CD14 level—that is, as $CD14^{-}$, $CD14^{int}$, or $CD14^{high}$—and confirmed that $CD14^{high}$ aPMN-MDSCs are associated with negative clinical outcomes [123]. However, a prospective analysis of peripheral blood neutrophils and PMN-MDSCs ($CD66b^{+}CD11b^{+}CD15^{+}CD14^{-}$) contradicted these results [124]. The researchers found that patients with NSCLC had a higher proportion of PMN-MDSCs and neutrophils than healthy individuals ($p < 0.0001$). Moreover, patients with low PMN-MDSCs had better OS (22.1 (95% CI: 4.3–739.7) months) than those with high PMN-MDSCs (9.3 (95% CI: 0–18.8) months). Hence, based on these contrasting findings, it is unclear whether CD14 can serve as a marker for PMN-MDSCs. These discrepant findings may be attributable to multiple unknown types of PMN-MDSCs and their different levels in peripheral blood versus tumor tissue. The observation that patients with low PMN-MDSCs had better OS was also supported by another study comprising a single-cell RNA sequencing analysis of tissue biopsy samples from 42 patients with stage III/IV NSCLC. The study identified two distinct clusters of TANs [99], both of which expressed various potential PMN-MDSC-related genes, including lipoxygenase 1 (*LOX-1*); thus, it demonstrated that PMN-MDSCs are present in tumors and challenging to distinguish from TANs. Another study found that neutrophils were more abundant in human lung SqCC than in lung adenocarcinoma.

3.3. Tumor-associated neutrophils

TANs were originally identified in mouse tumors and, similar to macrophages, are classified into two potential phenotypes—namely, antitumor N1 and pro-tumor N2—which have distinct functional cytokine and gene expression profiles [100–102,140]. The TME can affect the balance of N1 and N2 subpopulations through the secretion of cytokines, including TGF-β, IL-6, G-CSF, and IL-35, which induce pro-tumor polarization, while IFN-β and IL-12 induce antitumor polarization [69,84,141,142] (Fig. 2). In the TME, tumor cells attract TANs using chemokines such as CXCL8, which promotes CXCR1 and CXCR2 expression, contributing to angiogenesis and tumor progression [84,140]. TANs can also regulate other lymphocytes. N2 TANs can inhibit natural killer (NK) cell tumor cell killing function, thus promoting tumor metastasis [143,144] and promoting tumor growth by recruiting regulatory T cells through the secretion of C—C motif chemokine ligand 17 (CCL17) [145,146]. In contrast, N1 TANs recruit and activate $CD8^{+}$ T cells by producing chemokines, thus providing antitumor effects [147–150]. In a recent study, neutrophils were divided into five subpopulations in humans (hN1–5) and six subpopulations in mice (mN1–6) by spectral clustering [16]. Ratios of these subpopulations differed significantly between tumors and normal lung tissue; mN1s and mN2s were enriched in healthy lungs, while mN4s and mN5s were more abundant than mN1s and mN2s in tumor tissue, and mN3s and mN6s were only present in tumor tissue. The hN1 and mN1 subtypes expressed many typical neutrophil markers (i.e., MMP8, MMP9, S100A8, S100A9, and ADAM8), while the hN2 and mN2 cells expressed type I interferon-responsive genes, including interferon induced protein with tetratricopeptide repeats 1gene (*IFIT1*), interferon regulatory factor 7 gene (*IRF7*), and radical S-adenosyl methionine domain containing 2 gene (*RSAD2*). The hN5 and mN5 subtypes expressed the cytokines cystatin B (CSTB, a stefin that functions as an intracellular thiol protease inhibitor), cathepsin B (CTSB, a lysosomal cysteine protease with both endopeptidase and exopeptidase activity, which may play a role in protein turnover), CCL3 (which contributes to inflammatory responses by binding to the receptors CCR1, CCR4, and CCR5), and colony-stimulating factor 1 (CSF1, which encodes a cytokine that controls macrophage production, differentiation, and function). mN6 neutrophils are very similar to mN4 neutrophils in that both express hexosaminidase subunit beta (HEXB, the beta subunit of the lysosomal enzyme beta-

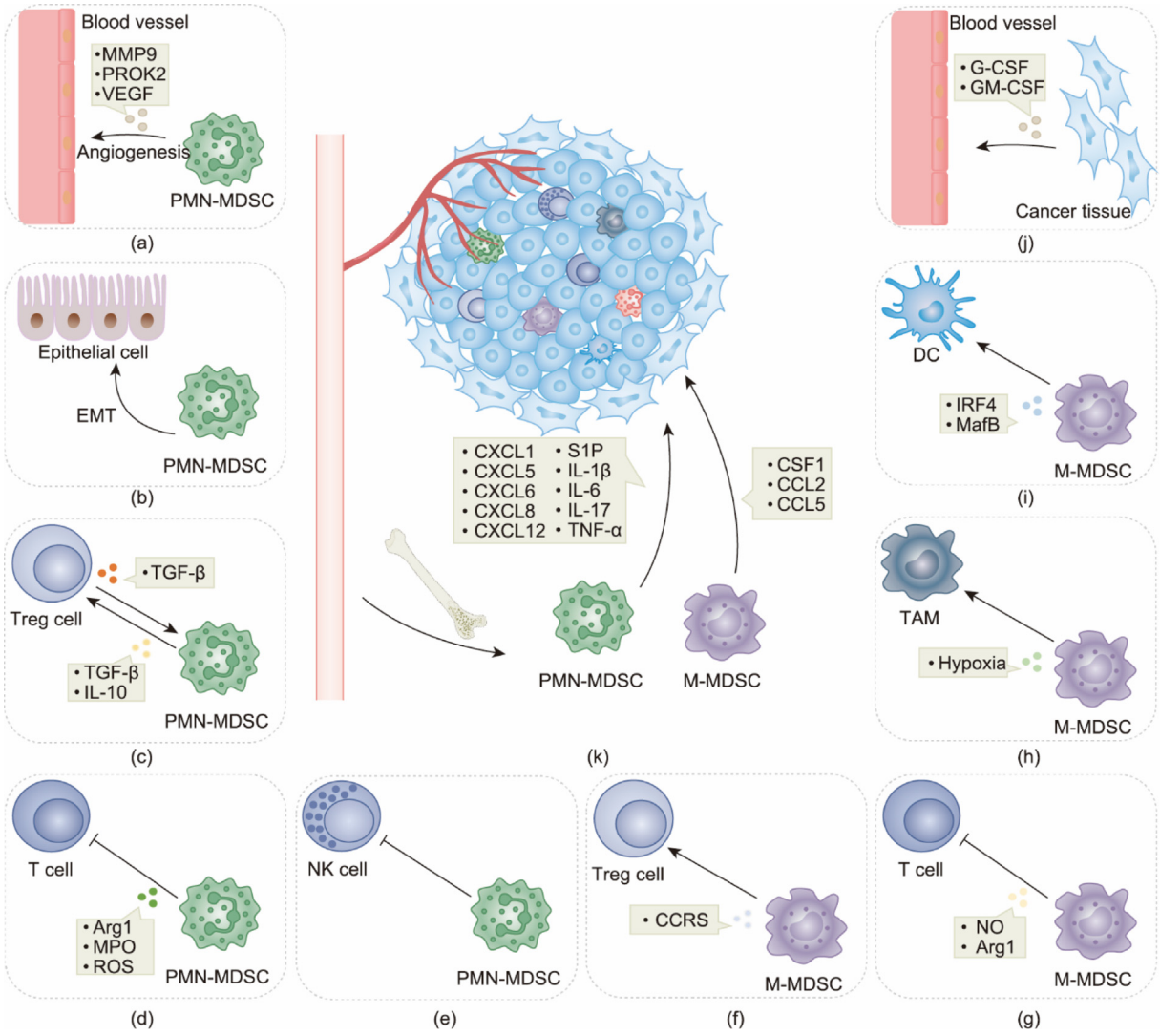

Fig. 1. The function of PMN-MDSCs and M-MDSCs in the TME. (a, b) PMN-MDSCs in tumors can promote intra-tumor angiogenesis through the MMP9, prokineticin 2 (PROK2), and VEGF pathways and can also promote epithelial-to-mesenchymal transition (EMT). (c) PMN-MDSCs can enrich regulatory T cells (Tregs) and promote tumor functions by releasing TGF-β and IL-10, and Tregs can positively regulate PMN-MDSCs. (d, e) PMN-MDSCs can inhibit T and NK cells. M-MDSCs can be attracted to and enriched in tumor tissues by CSF1, CCL2, and CCL5. (f, g) Like PMN-MDSCs, M-MDSCs can inhibit T cells and promote Treg activity. (h, i) In contrast, M-MDSCs can be directly transformed into tumor-associated macrophages (TAMs) and dendritic cells (DCs). IRF4: interferon regulatory factor 4; MafB: ZIP transcription factor B. (j) PMN-MDSCs and M-MDSCs can be released into the circulation from the bone marrow in response to G-CSF and GM-CSF secretion by some tumor tissues. (k) PMN-MDSCs and M-MDSCs are enriched in tumors by several specific types of chemokine. S1P: sphingosine-1-phosphate.

hexosaminidase, which catalyzes degradation of the ganglioside GM2 and of other molecules containing terminal *N*-acetyl hexosamines) and prothymosin alpha (PTMA, which is involved in negative regulation of apoptosis). Furthermore, mN6 neutrophils uniquely express ficolin B (Fcnb, which is involved in complement activation and the lectin pathway) and neutrophilic granule protein (Ngp, which is involved in negative regulation of angiogenesis and lymphangiogenesis).

As different neutrophil types have contrasting functions, artificial alteration of the abundances of neutrophil populations may have unexpected results; for example, inhaled Cowpea Mosaic Virus nanoparticles (CPMV-NPs) comprise 60 copies of self-assembling virus-like NPs that generate a 30-nm icosahedral structure composed of differently sized capsid protein units. It can inhibit the lung metastasis of certain cancers by increasing the frequency of $CD11b^{+}CD86^{high}$ tumor-infiltrating neutrophils (Table 6). $CD11b^{+}CD86^{high}$ neutrophils can kill tumor cells by releasing reactive oxygen intermediates, priming $CD4^{+}$ T cells and inducing them to a helper T (Th) 1 phenotype, and modulating NK cell function. Moreover, $CD11b^{+}CD86^{high}$ neutrophils can produce CXCR3, CXCL9, and CXCL10, which recruit $CD4^{+}$ and $CD8^{+}$ T cells and generate an antitumor immune microenvironment. This CPMV-NPs represent a new pathway that could be targeted for treating malignant tumors [151].

3.4. Low-density neutrophils

Numerous clinical trials have demonstrated that the numbers of circulating neutrophils are increased and that high NLR values are associated with aggressive outcomes in patients with lung cancer. The density gradient centrifugation of peripheral blood separates HDNs and LDNs, which are respectively found in the high- and low-density fractions [103,104,109–112,152,153]. HDNs account for approximately 95% of peripheral blood neutrophils and express CD66b, CD11b, CD15, CD16, and CD10 in humans and CD11b, Gr1, and Ly6G in mice; however, in tumor-bearing mice, the number of LDNs increases with tumor progression [154]. HDNs are reported to have antitumor effects, while LDNs have pro-tumor effects [152–

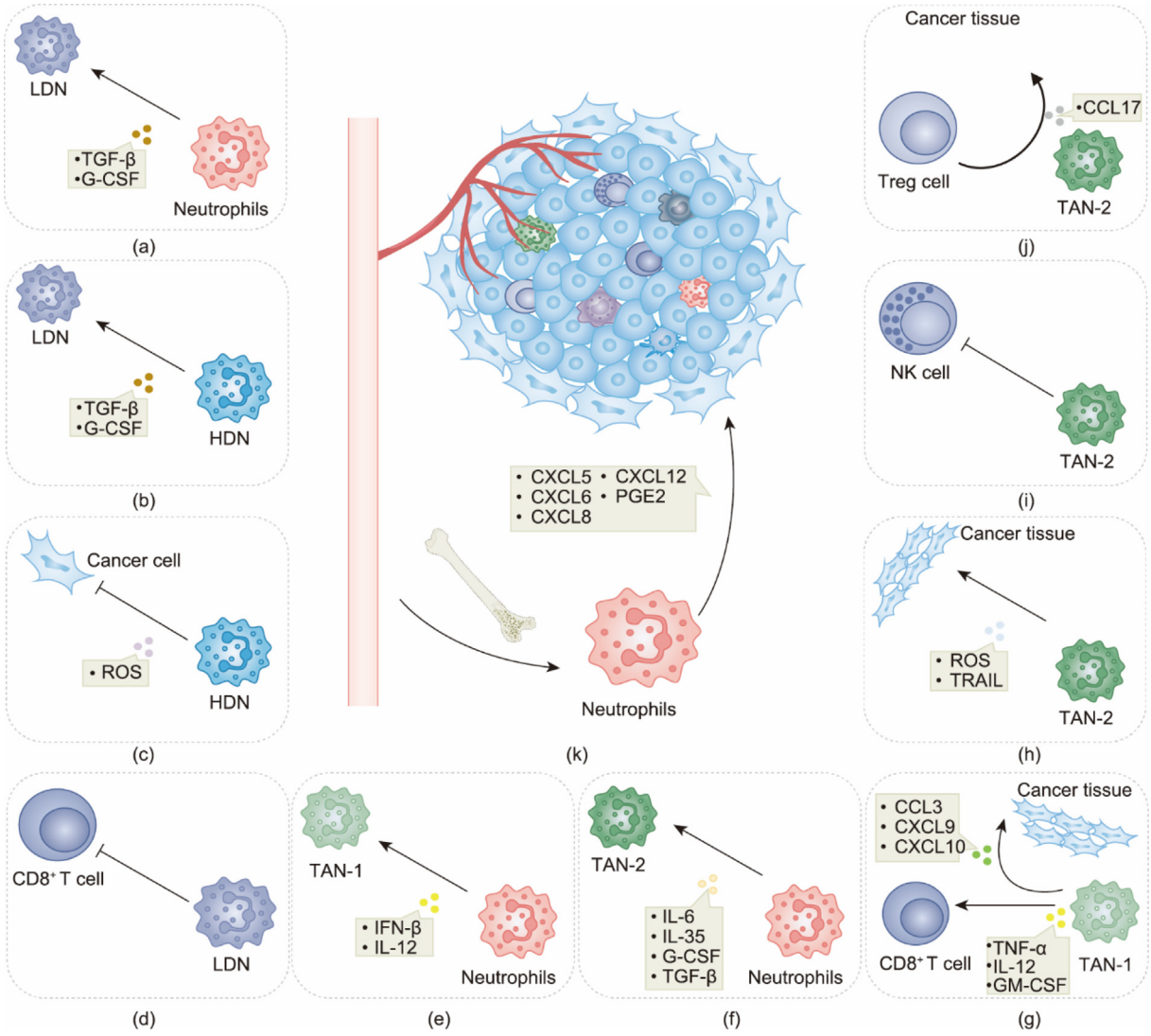

Fig. 2. Neutrophils in the TME. (a, b) Under the influence of TGF-β and G-CSF, neutrophils and HDNs can be converted to LDNs. (c) HDNs can directly inhibit tumor cells through ROS signaling, while (d) LDNs can indirectly promote tumor progression by suppressing $CD8^+$ T cells. (e, f) In the TME, neutrophils can be transformed into TAN-1 and TAN-2 subtypes under different conditions. (g–j) TAN-1 cells can recruit and activate $CD8^+$ T cells to inhibit tumor progression, while TAN-2 cells can indirectly promote tumor progression by inhibiting NK cells or recruiting Tregs, in addition to exerting direct tumor-promoting activity. (k) Neutrophils can be enriched in tumors by several chemokines, including CXCL5, CXCL6, CXCL8, CXCL12, and prostaglandin E2 (PGE2).

154]. *In vitro* experiments demonstrated that HDNs accumulate in the premetastatic lung and inhibit tumor cell implantation by releasing ROS; in tumor-bearing mice, tumors can be suppressed by HDN injection [154]. LDNs are a heterogeneous group associated with various diseases, including asthma, sepsis, rheumatoid arthritis, systemic lupus erythematosus, and cancers [104,105]. Furthermore, LDNs can promote tumor development by downregulating the expression of inflammatory factors and limiting $CD8^+$ T cell proliferation. LDNs lack specific biological markers and share the same surface markers as PMN-MDSCs and TANs. Moreover, like TANs, LDNs display pro-tumor properties in malignant tumors, making it difficult to distinguish these subgroups [106] (Fig. 2).

To clarify the specific role of LDNs in lung cancer, a clinical trial analyzed the composition of peripheral blood from patients with lung cancer and healthy subjects and found that LDNs were elevated in patients with advanced lung cancer and associated with poor clinical prognosis. Phenotypes of HDNs and LDNs were also characterized in patients with advanced lung cancer (stages IIIB–IV) by applying a unique, newly developed mass cytometry method; two HDN subpopulations ($CD66b^{high}CD10^{int}CXCR4^{int/low}PDL1^{low}$ and $CD66b^{high}CD10^{high}CXCR4^{int}PDL1^{low}$) were detected in both healthy individuals and patients, while one unique LDN subpopulation ($CD66b^{high}CD10^{low}CXCR4^{high}PDL1^{int}$) was exclusively found in patients with cancer [107]. In another study, a high-dimensional human cell surface marker screen identified 12 down-regulated and 41 up-regulated surface markers on LDNs compared with HDNs, and finally selected CD36, CD41, CD61, and CD226 as surface markers of LDNs [113]. Although this study identified new markers of LDNs, indicating that LDNs are a distinct neutrophil population, much remains unknown about this cell type, and further future exploration is warranted.

4. The role of neutrophils in lung cancer development and their corresponding nanotechnology therapeutic targets

4.1. The dual role of neutrophils in the development and progression of lung cancer and tumor-targeting nanotechnologies

There are diverse immune cells in the NSCLC TME, and flow cytometry has demonstrated that neutrophils are the dominant immune cell type in this context, accounting for almost 20% of

Table 6
Nanoparticle strategies to target neutrophils.

Type	Abbreviation	Targeting strategies
Cowpea Mosaic Virus nanoparticles	CPMV-NPs	Increasing the frequency of $CD11b^{+}CD86^{high}$ tumor-infiltrating neutrophils
Inhibitor of STAT3 encapsulated in nanoparticles	—	Inhibiting STAT3 and consequent immune activation
Polyethylene glycol (PEG) ylated liposome doxorubicin nanoparticles	PLD@NEs	Using neutrophils as carriers and increasing the concentration of inflammatory factors (CXCL1/KC) in tumor sites
Interlayer-crosslinked multilamellar vesicles	ICMV	Delivering sivelestat that is taken up by neutrophils and inhibits NETs formation
Silver nanoparticles decorated with PEGylated sialic acid	AgNPs	Recognizing L-selectin expressed on neutrophils, enabling them to accumulate at neutrophil-rich tumor sites and deplete neutrophils locally
Neutrophil-mimicking-nanoparticles	NM-NPs	High circulating tumor cells (CTC) captures ability, selectively neutralizes CTCs, and prevents the formation of a premetastatic niche when combined with carfilzomib
Solid lipid nanoparticles containing astaxanthin	AX-SLN	Reducing nuclear factor-κB (NF-κB) expression, resulting in antiproliferative, antioxidant, chemotherapeutic, and anti-inflammatory effects
Nanoparticles containing a paclitaxel core and an MMP9 and DNase I shell	mP-NPs-DNase/PTX	DNase I degrades NETs in tumor tissues, and the paclitaxel (PTX) core exerts a cytotoxic effect on tumor cells
Hypoxia-inducible factor-1α small interfering Ribonucleic Acid(siRNA)-loaded superparamagnetic iron oxide-trimethyl chitosan-hyaluronate nanoparticles	—	Inhibiting hypoxia-inducible factor-1α (HIF-1α)/COX2/PGE2/prostaglandin E2 recepter 4 (EP4) signaling pathways, which in turn inhibits tumor cell proliferation, migration, and invasion
Active-targeted hyaluronate recoated N,N,N-trimethyl chitosan nanoparticles used to deliver siRNAs	siRNA-loaded HA-TMC NPs	Targeting IL-6 and STAT3 resulted in decreased proliferation, migration, colony formation, and angiogenesis in cancer
5-Hydroxytryptamine assembled onto nanoparticles containing photosensitizers and Zileuton	HZ-5 NPs	Inhibiting neutrophil-mediated lung metastasis through the sustained release of Zileuton

all $CD45^{+}$ cells. Myeloid lineage cells represent > 50% of tumor-infiltrating $CD45^{+}$ cells, while the percentage of macrophages is significantly lower. B cells comprise only 5% of $CD45^{+}$ cells in NSCLC. Combined T cell receptor (TCR)-b sequencing and experimental data indicate that the proportion of T cells in lung cancer varies greatly [155]. Neutrophils have essential roles in lung cancer occurrence and development. Cancer is a specific type of inflammatory condition [156] in which endothelial cells are exposed to products of inflammation, such as ROS and reactive nitrogen species produced by neutrophils, leading to the increased oxidation of proteins, lipids, and nucleic acids. If the proteasome cannot degrade those oxidized proteins, advanced glycation end-products accumulate and activate receptors for advanced glycation end-products (RAGE), resulting in tumor cell proliferation and carcinogenesis [97]. In non-small cell carcinomas and adenocarcinomas, basal RAGE expression is remarkably high, but it is rapidly down-regulated on malignant transformation, and this is associated with the highly aggressive phenotypes of these types of lung cancer [157].

Matrix metalloproteinases (MMPs), a family of zinc-dependent endopeptidases, can degrade basement membrane, the ECM, and non-matrix substrates, thereby acting as an angiogenic switch and contributing to tumorigenesis and tumor progression [158,159]. Neutrophils also contribute to the regulation of tumor angiogenesis, which is pivotal to the progression of early-stage tumors. The STAT3 signaling pathway activates neutrophil pro-angiogenic functions by controlling the transcription of vascular endothelial growth factor A, fibroblast growth factor 2, and MMP9 (Fig. 3). An inhibitor of STAT3 was encapsulated in NPs and used to treat a murine *HER-2*$^{+}$ breast cancer model, resulting in STAT3 inhibition and consequent immune activation via an increase of IFN-γ, GM-CSF, p- signal transducer and activator of transcription (pSTAT)-1, IL-15, IL-2, and IL-12b in the TME, which activated $CD8^{+}$ T cells and decreased tumor angiogenesis, tumor growth, and metastasis [160] (Table 6). MMP9 controls late tumor angiogenesis [161], and bone-marrow-derived neutrophils are the primary source of MMP9 in tumor-bearing lung tissues following tumor cell inoculation. An orthotopic lung cancer model showed that MMP9 promotes tumor cell survival at a very early stage of tumor establishment; however, its inability to influence tumor size suggests that its effect on subsequent tumor growth is limited [162]. When neutrophils are activated or destroyed, proteinases stored in neutrophil granules are released. NE, which is encoded by *Elane*, is among the most potent neutrophil proteinases; it can degrade the ECM and may contribute to lung cancer development. Molecular genetics analysis has shown that two single nucleotide polymorphisms (–903 T and –741G) in the *Elane* promoter might accelerate lung cancer development due to an imbalance of the α1- antitrypsin (AT)/NE system [163]. NE can also promote lung cancer cell growth and proliferation by degrading insulin receptor substrate 1 (IRS1), which is stored in the endosomal compartment in tumor cells. Hence, NE degradation of IRS1 must occur within cells. During NE degradation of IRS1, the interaction between phosphatidylinositide 3-kinases (PI3K) and platelet-derived growth factor receptor (PDGFR) is increased, modulating the P13K axis toward promoting cancer cell proliferation; however, NE cannot induce lung cancer cell proliferation directly [164]. The MiR-30 family is an important regulator of signaling networks and suppresses the expression of molecules involved in cell proliferation, such as epidermal growth factor receptor (EGFR), type 1 insulin-like growth factor receptor (IGF1R), IRS1, and *E2F* transcription factor 7 (E2F7); it may play a pivotal role in microRNA (miRNA) replacement nanomedicine therapy [165].

Neutrophils induce tumor cell detachment by altering cell-to-cell contact, which involves several membrane-bound molecules, including tumor necrosis factor-α (TNF-α)/TNF-α receptor inhibitor, intercellular adhesion molecule-1 (ICAM-1)/lymphocyte function-associated antigen-1 (LFA-1), interleukin-1α (IL-1α)/IL-1α receptor, and NE [166] (Fig. 4). In patients with adenocarcinoma bronchioloalveolar carcinoma (BAC) features, neutrophil tumor shedding is crucial to aerogenous cancer cell spread [167]. Hepatocyte growth factor (HGF), a pleiotropic cytokine produced by neutrophils, also contributes to the aerogenous spread of BAC by activating cellular-mesenchymal epithelial transition factor (c-met). HGF can be detected in bronchoalveolar lavage fluid from most patients with BAC but not in healthy controls, which indicates that HGF is produced locally. The TME of BAC can attract neutrophils from peripheral blood, and neutrophil-derived HGFs induce BAC tumor cell migration and promote cancer cell spread [168].

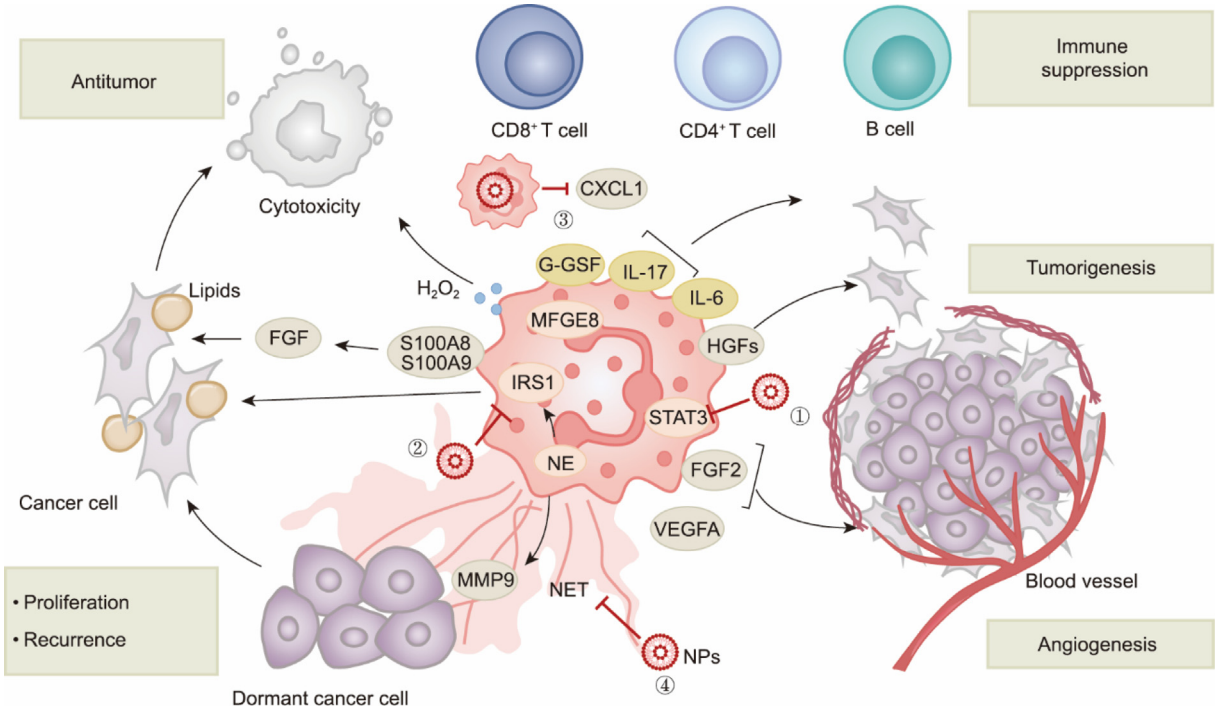

Fig. 3. The dual role of neutrophils in both lung cancer development and progression and their counterpart nanotherapeutics. Neutrophils play important roles in lung cancer occurrence and development by secreting MMP9, VEGFA, hepatocyte growth factors (HGFs), NE, and other cytokines; activating STAT3 and P13K signaling; and promoting the formation of NETs. Neutrophils play antitumor roles mainly via neutrophil cytotoxicity and by increasing adhesion between tumor cells and ECM proteins. ① Inhibitor of STAT3 encapsulated in NPs; ② miRNA replacement nanomedicine; ③ PLD@NEs; ④ a plasmonic gold blackbody (AuPB) core and a mesoporous polydopamine (mPDA) shell nanoparticles (AuPB@mPDA). IRS1: insulin receptor substrate 1; MFGE8: milk fat globule epidermal growth factor 8.

Genomic and transcriptomic technologies have enabled a better understanding of the molecular basis of lung cancer and have revealed that inflammation is a prominent characteristic of lung cancer. As the dominant immune cells in lung cancer, neutrophils may be able to suppress lymphocytes in SqCC [169]. A *Kras* mutant lung cancer animal model showed that IL-17A recruits neutrophils to tumor sites by inducing expression of the proinflammatory cytokines IL-6, G-CSF, and MFGE8, as well as the chemokine CXCL1, leading to reduced recruitment of CD4 T cells, CD8 T cells, and B cells. PEGylated liposome doxorubicin NPs (PLD@NEs)—a model chemotherapeutic nano-drug using neutrophils as carriers—significantly increase the concentration of inflammatory factors (CXCL1/keratinocyte chemoattractant (KC)) in tumor sites, which is essential to PLD@NEs infiltration [170] (Table 6). The neutrophil count is negatively correlated with T cell count in patients with NSCLC [171]; furthermore, neutrophil and $CD8^+$ cell content are negatively correlated in cancer but not in adjacent normal tissues, suggesting that this phenomenon is tumor-specific.

Moreover, a negative correlation between neutrophils and $CD4^+$ lymphocyte content—particularly Th1 and Th17 subsets—has been detected [155]. Lung adenocarcinoma with a mutation of serine/threonine kinase 11 (*STK11*), a tumor suppressor gene, exhibits a marked reduction of dendritic cells, NK T cells, and macrophages. Neutrophil degranulation is a potential immunosuppressive mechanism associated with *STK11* mutation; however, the evidence supporting this hypothesis is limited to proteomics data [172].

Tumor recurrence seriously affects the survival of patients with lung cancer, and neutrophils can reactivate dormant tumor cells by releasing proinflammatory S100A8/S100A9 proteins under the control of stress hormones. S100A8/S100A9 release activates myeloperoxidase (MPO), leading to the accumulation of oxidized lipids in neutrophils. Releasing these lipids from neutrophils activates the FGF pathway, allowing dormant tumor cells to form new tumors; hence, high S100A8/S100A9 expression results in early lung cancer recurrence [173] (Fig. 3).

AuPB@mPDA, a nanoplatform for efficient loading and the photoregulated release of DNase I, can disrupt NET formation, thereby enhancing the therapeutic efficacy of tumor immunotherapy and moderating metastatic spread [174] (Fig. 3). In response to inflammatory cues to trap and kill pathogens, DNA webs derived from neutrophils can awaken dormant cancer cells. Two NET-associated proteases, NE and MMP9, sequentially cleave and remodel laminin, resulting in the proliferation of dormant cancer cells by activating $\alpha 3\beta 1$ integrin signaling in response to sustained inflammation [175]. NE is involved in NETs formation, which facilitates lung cancer progression. Sivelestat, a small-molecule NE inhibitor, can function *in vitro* but does not work *in vivo* [176]. A new kind of lipid-based NP-mediated delivery system was found to improve sivelestat efficiency. In a mouse model, interbilayer-crosslinked multilamellar vesicles delivered sivelestat, which was taken up by neutrophils and inhibited NETs formation [177] (Table 6).

As mentioned above, neutrophils comprise heterogeneous populations with pro-tumor and antitumor functions. Neutrophils play their antitumor roles mainly via neutrophil cytotoxicity. H_2O_2, a type of ROS secreted by neutrophils, is an essential mediator of tumor cell killing that induces Ca^{2+} channel activation, resulting in Ca^{2+} influx into tumor cells [65]. Transient receptor potential cation channel, subfamily M, member 2 (TRPM2), an H_2O_2-dependent Ca^{2+}-permeable channel, is relatively likely to be found on tumor cells that have undergone epithelial-to-mesenchymal transition (EMT) but is reduced on cells that have undergone mesenchymal-to-epithelial transition (MET). Cells expressing high levels of TRPM2 are more susceptible to neutrophil cytotoxicity, indicating that EMT promotes tumor cell sensitivity to neutrophil cytotoxicity [178]. Neutrophils also exert antitumor effects via

tumor necrosis factor-related apoptosis-inducing ligands (TRAILs). In response to IFN-γ stimulation, neutrophils exert their cytotoxic function by elevating TRAIL expression and release, which promotes cancer cell apoptosis [179].

The loss of testosterone can impair neutrophil antitumor function. In castrated mice, *Cxcr2* messenger RNA (mRNA) levels are reduced, while *Cxcr4* and *Vla-4* are increased, which is opposite to the situation during neutrophil maturation and leads to an increased likelihood of tumor formation in the lung [180]. RAGE plays a pro-tumor role under inflammatory conditions; however, it also has antitumor functions. When RAGE on tumor cells interacts with and is recognized by cathepsin G on neutrophils, the neutrophil-mediated killing of tumor cells is activated [181]. These phenomena illustrate the complex dual role of neutrophils in cancer.

The interaction between C-type lectin receptors in neutrophils and tumor Nidogen-1 can also enhance neutrophil cytotoxicity toward mouse tumor cells [182]. In addition to MMP9, neutrophils produce MMP8, which has an antitumor function. MMP8 has been shown to increase the adhesion between tumor cells and ECM protein levels, thereby reducing the invasive ability of tumor cells through Matrigel and inhibiting tumor metastasis, as well as regulating the inflammatory responses induced by carcinogens [183].

Neutrophils interact with other immune cells, as well. TANs isolated from lung tumors can activate T cell proliferation, increasing costimulatory molecule expression on the surface of neutrophils. This positive-feedback loop demonstrates that TANs exert their antitumor effects by activating T cell responses [184]. The H_2O_2 produced by neutrophils is vital in killing cancer cells and enables neutrophils to inhibit cancer cell seeding in the lung [82]. A core–shell supramolecular hybrid nano-gel has been designed to mimic the function of neutrophil lysosomes; it can be used to treat tumors through a cascade reaction involving superoxide dismutase and chloroperoxidase [185].

4.2. Nanotherapeutics targeting the mechanisms underlying neutrophil promotion of lung cancer metastasis

Lung cancer metastasis is an important factor affecting patient prognosis. Neutrophils accelerate lung cancer metastasis through two main pathways. The first pathway is a contact-dependent mechanism. Selectins expressed on endothelial cells (E-selectin), platelets (P-selectin), and leukocytes (L-selectin) bind to selectin ligands, such as sialyl Lewis-a (sLe^a) and sialyl Lewis-x (sLe^x), expressed on circulating tumor cells (CTCs), increasing the adhesion between cancer cells and distant organs [186] (Fig. 4). *Salmonella* exhibits antitumor behavior through various mechanisms; however, neutrophils recruited into tumor sites by *Salmonella* directly eliminate the bacteria, limiting the antitumor efficacy of *Salmonella*; hence, a neutrophil-depleting strategy is

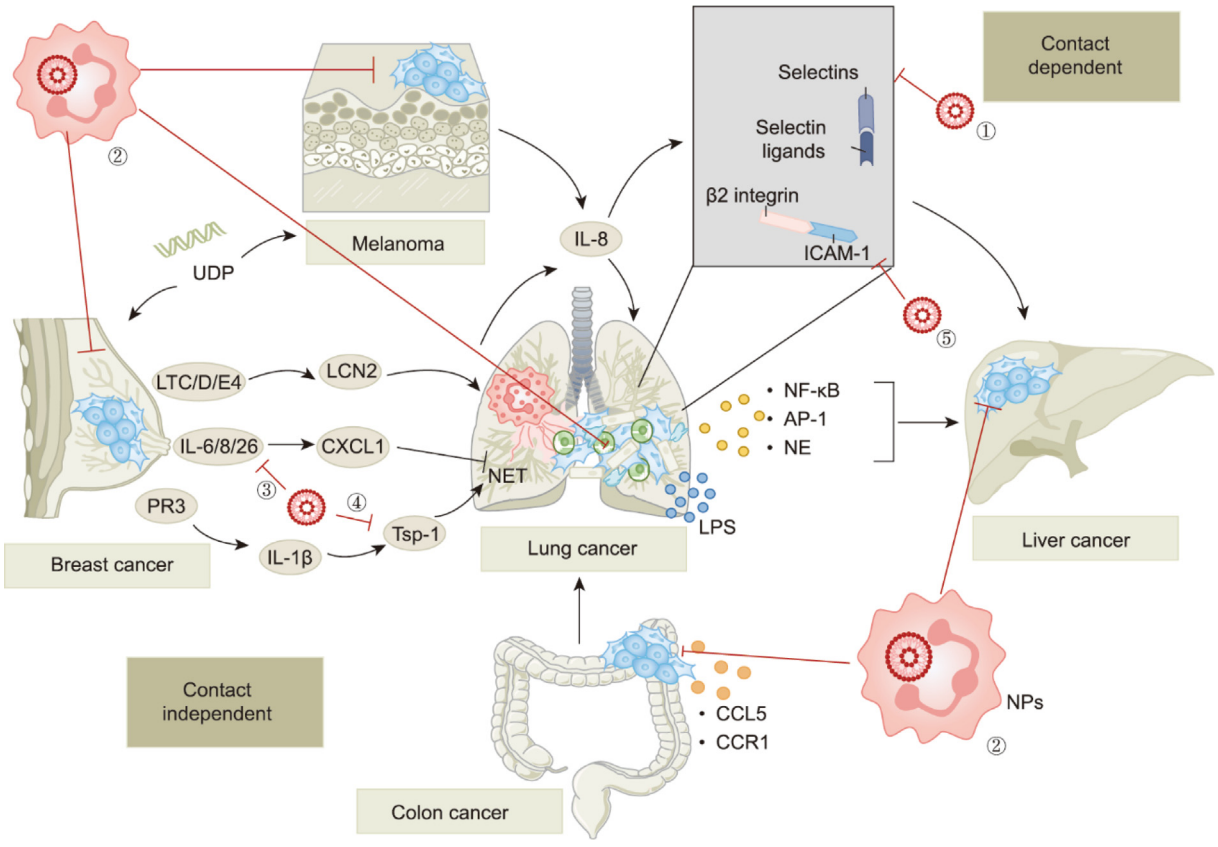

Fig. 4. Neutrophil promotion of lung cancer metastasis and lung metastasis of breast, colon, and liver cancers, and melanoma, as well as their counterpart nanotherapeutics. Neutrophils promote lung cancer metastasis through contact-dependent and -independent pathways. Systemic inflammation activates neutrophils and promotes adhesion between cancer cells and other cells, spreading cancer cells. NETs can trap circulating tumor cells (CTCs), thereby increasing adhesion between tumor cells and distant organ sites. The NE–IL-8–mitogen-activated protein kinase (MAPK) pathway accelerates tumor metastasis. The PR3–IL-1β–NETs cascade; IL6, IL8, and CXCL1 pathway; and leukotrienes C4, D4, and E4 (LTC/D/E4) and lipocalin 2 (LCN2) promote the lung metastasis of breast cancer by interacting with neutrophils. IL-8 increases ICAM-1 expression on melanoma cells and β2 integrin levels on neutrophils, enhancing cell adhesion and resulting in lung metastasis development. Loss of SMAD family member 4 (SMAD4) promotes lung metastasis of colorectal cancer through an accumulation of $CCR1^+$ TANs. ① AgNPs; ② NM-NPs; ③ siRNA-loaded HA-TMC NPs; ④ sphingomyelin nanosystems (SNs) were designed to associate with the cell-binding domain adhesive peptide (4N1K) peptide (SNs-4N1Ks NPs); ⑤ anti-ICAM-NPs. CCR1: C—C motif chemokine receptor 1; Tsp-1: thrombospondin-1; AP-1: activator protein-1.

required to improve its efficacy. Silver NPs (AgNPs) decorated with PEGylated sialic acid were designed to recognize the L-selectin expressed on neutrophils, enabling the AgNPs to accumulate at neutrophil-rich tumor sites and deplete neutrophils locally. These AgNPs were then applied in combination with *Salmonella* in a neutrophil-depleting model to treat tumors. Moreover, AgNPs have intrinsic antitumor activity; hence, these NPs can exert their antitumor effects both by improving the efficacy and safety of *Salmonella* and through neutrophil depletion [187] (Table 6).

Lipopolysaccharide-induced systemic inflammation may activate neutrophils and influence cell adhesion, leading to cancer cell spread. Photodynamic therapy (PDT)-induced acute inflammation in deep tumor tissues can be used as tumor therapy. PDT-induced inflammation accelerates neutrophil infiltration in tumor tissues, enabling nanotherapeutics to incorporate neutrophils during the design of drug delivery systems [188]. In more than 30% of patients with lung cancer metastasis, the metastasis is to the liver. Activated neutrophils within inflamed liver sinusoids can facilitate CTC binding with liver sinusoids and mediate tumor cell arrest. Interactions among selectin-sLe^x-neutrophils increase the liver metastasis of lung cancer [189]. The molecular mechanism underlying neutrophil interaction with cancer cells has also been explored. Under inflammatory conditions, neutrophils are activated by inflammatory stimuli, potentially leading to endothelial cell dysfunction, with changes in cell adhesion molecule (CAM) expression on the neutrophils surface. Mac-1, a type of CAM, changes its expression on the surface of neutrophils, affecting their adherence and migratory abilities within potential metastatic tissues.

Moreover, the expression of the binding ligand of Mac-1, ICAM-1, is increased on CTCs, and interaction between the Mac-1 expressed on neutrophils and the ICAM-1 expressed on CTCs promotes cancer cell arrest in distant organs, leading to distant metastasis of lung cancer [70]. Based on this mechanism, neutrophil-mimicking (NM)-NPs were designed by coating neutrophil membranes onto a poly (lactic-*co*-glycolic acid) (PLGA) surface to preserve the bio-binding ability of the neutrophils. These NM-NPs have high CTC-capture ability. Furthermore, combining NM-NPs with carfilzomib, a second-generation proteasome inhibitor, selectively neutralized CTCs and prevented the formation of a premetastatic niche in a breast cancer murine model [190] (Fig. 4 and Table 6). Neutrophils can also accelerate liver metastasis through the formation of NETs. Extracellular RNAs released from Lewis lung carcinoma cells enhance the formation of NETs by activating epithelial cells and increasing their IL-1β release and the CAM-1 expression on their surface [191]. A mathematical model also showed that NETs play important roles in regulating lung cancer invasion and metastasis [192]. Under conditions of severe postoperative infection, NETs can trap CTCs, increasing the adhesion between tumor cells and distant organ sites and facilitating lung cancer metastasis [193]. Neutrophils have also been used as carriers of liposomes containing paclitaxel (PTX). Under inflammatory-factor stimulation, NETs and PTX liposomes are released from neutrophils and then enter glioma tumor cells, where PTX has cytotoxic effects and inhibits tumor development [194].

The second pathway is contact independent. IL-8, a member of the α-chemokine family and a highly potent neutrophil chemoattractant released by tumor cells, is associated with cancer progression and metastasis. In response to IL-8, neutrophils release enzymes that can hydrolyze ECM components and remodel ECM, accelerating tumor angiogenesis and metastasis [195]. Neutrophils can also induce IL-8 release. An experiment using human NSCLC A549 cells showed that NE could dose-dependently increase IL-8 release, and that the underlying mechanism mainly involves the mitogen-activated protein kinase (MAPK) pathway. p38 MAPK, an activator of many transcription factors, can be activated by NE and upregulates nuclear factor-κB (NF-κB) and activator protein 1 (AP-1); the latter binds with the IL-8 promoter and increases its transcription [196]. Nuclear translocation of NF-κB is involved in carcinogenesis via activating inflammatory signaling pathways. Astaxanthin (AX) can reduce the oxidative imbalance in inflammation. To reduce NF-κB expression, solid lipid NPs containing AX (AX-SLN) were prepared and resulted in antiproliferative, antioxidant, chemotherapeutic, and anti-inflammatory effects in breast-cancer-bearing rats [197] (Table 6).

5. Neutrophils enhance both the lung metastasis of other cancers and their counterpart nanotherapeutics

5.1. Neutrophils promote breast cancer metastasis to the lungs

The lung is the preferred metastatic organ for breast cancer, melanoma, and colon cancer, and neutrophils play critical roles in the metastasis of these cancers to the lung. Primary tumors may change the paracrine characteristics of distant organs to facilitate the metastatic colonization of resident cancer cells in structures referred to as premetastatic niches. Premetastatic niche formation is essential to colonize cancer cells in distant organs, and lung mesenchymal stromal cells (LMSCs) contribute to establishing such niches. Complement 3 (C3) is upregulated in LMSCs and can recruit neutrophils and promote the formation of NETs. Th2 cytokines can induce C3 under STAT6 regulation; hence, the STAT6–Th2–C3–NETs cascade promotes breast cancer metastasis to the lungs [198] (Fig. 4). A well-designed nanocarrier named mP-NPs-DNase/PTX, which contains a PTX core and an MMP9 and DNase I shell, can release DNase I to degrade NETs in murine lung cancer and breast cancer tumor tissues, while the PTX core exerts a cytotoxic effect on tumor cells; together, these two aspects function to inhibit tumor growth and distant metastasis [199] (Table 6). Eukaryotic translation initiation factor 4E (eIF4E) is a well-known translation control factor whose phosphorylation promotes EMT and the translation of pro-survival and pro-invasion mRNAs, such as B-cell lymphoma-2 (BCL2), myeloid cell leukemia-1 (MCL1), SNAIL, and MMP3 [200,201], thereby enhancing cancer invasion and metastasis; BCL2 and MCL1 are also anti-apoptotic proteins [202]. In mouse breast cancer models, eukaryotic initiation factor 4E (eIF4E) mutation leads to the loss of eIF4E phosphorylation, resulting in decreased expression of BCL2 and MCL1, influencing neutrophil survival in the premetastatic niche, and preventing metastatic progression *in vivo*. These results demonstrate that neutrophils in the lung favor metastasis to the lung [203] and support the initiation of lung metastasis of cancers.

In mouse breast cancer models, CD11b$^+$Ly6G$^+$ neutrophils are the most frequent immune cells in the premetastatic lung. Moreover, neutrophils accumulate earlier than cancer cells [71], and the depletion of neutrophils within premetastatic niches decreases spontaneous metastasis, indicating that neutrophils promote breast cancer metastatic initiation. CD11b$^+$Ly6G$^+$ neutrophils are essential for the internalization of anti-CD11b-coated NPs and thus provide a platform for gold nanorods-anti-CD11b therapy [204]. Neutrophil-derived factors—including the lipids leukotriene B4 and the cysteinyl leukotrienes C4, D4, and E4 (LTC/D/E4), which are produced by the arachidonate 5-lipoxygenase (Alox5)—mediate metastatic initiation by providing a selective proliferative advantage to cancer cells and transforming them into highly metastatic cells, by enhancing their metastatic competence [71]. Chronic exposure to nicotine also contributes to premetastatic niche formation by recruiting pro-tumor N2 neutrophils. Within the lungs, N2 neutrophils secrete the glycoprotein STAT3-activated lipocalin 2 (LCN2), which induces breast cancer cell EMT, thereby promoting colonization and metastatic outgrowth

[72]. CD140a$^+$ mesenchymal cells in the lung produce prostaglandin E2 (PGE2), which binds with the PGE2 receptors expressed on neutrophils in lung tissue, leading to lipid accumulation in the lung and the reduction of adipose triglyceride lipase expression. PGE2 expression by mesenchymal cells can, in turn, be induced by the cytokine IL-1β, which is produced by neutrophils. Lipids from neutrophils promote the metastasis of breast tumors to the lungs [205]. Hypoxia-inducible factor-1α (HIF-1α) overexpression in the TME contributes to tumor cell growth. HIF-1α siRNA-loaded superparamagnetic iron oxide-trimethyl chitosan (TMC)–hyaluronate (HA) NPs were designed to treat breast cancer, colorectal cancer, and melanoma cell lines, and were found to significantly inhibit the HIF-1α/COX2/PGE2/EP4 signaling pathways, thereby inhibiting tumor cell proliferation, migration, and invasion [206] (Table 6).

Triple-negative breast cancer (TNBC), which is one of the most common types of breast cancer, is characterized by an inflammatory TME that is mainly composed of cytokines and chemokines. This inflammation stimulates the growth and distant metastasis of cancer cells. IL-26 is a cytokine produced by TNBC cells that belongs to the IL-10 cytokine family; it has an amphipathic nature that allows it to bind to extracellular DNA—particularly NETs. The interaction between NETs and IL-26 induces the expression of other cytokines, such as IL-6/IL-8 and CXCL1, which elicits inflammation in the TME, activating Janus-family tyrosine kinase (JAK)–STAT3 signaling and promoting cancer progression and TNBC metastasis to the lung [207]. Active-targeted HA-recoated N,N,N-TMC NPs were used to deliver siRNAs targeting IL-6 and STAT3. The NPs showed high IL-6/STAT3 inhibition capacity, which decreased proliferation, migration, colony formation, and angiogenesis in breast, colorectal, and melanoma cancer cell lines [208]. Moreover, tumor-secreted protease cathepsin C (CTSC) promotes neutrophil recruitment and the formation of NETs, resulting in enhanced breast-to-lung metastasis. The underlying mechanism involves the CTSC activation of neutrophil membrane-bound proteinase 3 (PR3) through enzymolysis, and the subsequent PR3-mediated activation of IL-1β release and NF-κB expression, as well as the upregulation of IL-6 and CCL3, resulting in neutrophil recruitment. The CTSC–PR3–IL-1β axis also activates p38 and subsequently induces neutrophil ROS production and NETs formation, leading to thrombospondin-1 (Tsp-1) degradation and the metastatic growth of breast cancer cells in the lungs [73]. Sphingomyelin nanosystems (SNs) were designed to associate with the cell-binding domain adhesive peptide (4N1K) peptide, a ten-amino-acid peptide derived from the Tsp-1 protein. SNs-4N1Ks inhibit cancer cell colony formation [209]. Furthermore, breast cancer cells can induce NETs formation without infection and promote breast cancer cell invasion and migration [210]. In late-stage breast cancer, the formation of NETs is associated with venous thrombi in the lung, which provide an environment conducive to lung colonization by cancer cells [211].

5.2. Neutrophils promote melanoma and colorectal cancer metastasis to the lungs

The extracellular nucleotide uridine diphosphate (UDP) is a signaling molecule that binds with and activates its receptor, purinergic receptor P2Y, gprotein-coupled,6 (P2y6), to promote breast cancer and melanoma invasion. Moreover, *P2y6* knockout was found to significantly reduce neutrophil infiltration and slightly alter NK cell, macrophage, T cell, and dendritic cell infiltration in melanoma premetastatic niches, altering the immune cell composition in the TME (Fig. 4). This finding underlines the pivotal role of neutrophils in promoting tumor metastasis [212]. Under inflamed conditions, neutrophils recruited to the lungs degranulate azurophilic granules to release the serine proteases, cathepsin G, and elastase, causing the hydrolysis of the antitumorigenic factor Tsp-1. This finding provides a new perspective on how neutrophil-mediated lung inflammation contributes to melanoma metastasis to the lung [74]. Anti-CD47 antibodies can improve cancer cell phagocytosis by inhibiting CD47 from binding with its ligands, such as Tsp-1 and signal regulatory protein α [213]. IL-8 also plays an important role in melanoma metastasis. Cancer cells injected in the lungs secrete IL-8, which increases β2 integrin expression and activates neutrophils. The ICAM-1 expressed on melanoma cells interacts with the β2 integrin expressed on neutrophils, enhancing cell adhesion, which results in an increased accumulation of cancer cells in the endothelium and retention in the lungs, thereby increasing lung metastasis development [214]. Anti-ICAM NPs have been exploited to deliver inflammation inhibitors and therapeutic enzymes to epithelial cells, resulting in decreased neutrophil infiltration [215].

Approximately 5%–15% of colorectal cancers metastasize to the lungs; hence, the molecular mechanisms involved have attracted the attention of researchers. Patients with colorectal cancer and deficiency of contraction of Sma and Mad 4 (SMAD4) (a cancer suppressor) have high CCL15 expression and CCR1$^+$ TAN accumulation in lung metastases, demonstrating that the loss of SMAD4 promotes the lung metastasis of colorectal cancer through the accumulation of CCR1$^+$ TANs via the CCL15–CCR1 axis [216]. In patients with differentiated thyroid carcinoma and distant metastasis, an NLR > 3 is strongly associated with poor cause-specific survival [217]. In human anaplastic thyroid carcinoma (ATC) cells, neutrophil gelatinase-associated lipocalin (NGAL, which belongs to a large family of lipocalins and is also known as LCN 2) [218] mediates NF-κB oncogenic activity, as well as influencing ATC cell invasion ability by increasing MMP9 enzymatic activity. cells with neutrophil gelatinase-associated lipocalin (NGAL) knocked down exhibit a reduced ability to form lung metastasis in nude mice [219].

NETs can also trap the CTCs generated by lung cancer, carrying them to distant organs, increasing their contact with metastatic tissues, and facilitating their avoidance of destruction by the immune system, thereby promoting lung cancer metastasis (Fig. 4). For the lung metastasis of breast cancer, gastric cancer, and colorectal cancer to occur, the formation of a pre-metastasis niche and the survival and growth of metastatic tumor cells are important [210,220,221].

6. Nanotechnology targeting neutrophils in cancer therapy

6.1. Nanotechnology targeting neutrophils in conventional cancer treatment and detection

Aside from the use of neutrophils as a prognostic or predictive biomarker for patients with cancer, the multiple functions of neutrophils in cancer biology make neutrophils useful as therapeutic targets. Nanotechnology is used to engineer, measure, assemble, and manufacture materials at a nanometer scale (1–100 nm) [222]; hence, nanotechnology deals with and takes advantage of small things, and its precision and controllability in both drug delivery and biosensing are unique [223–227]. Due to the natural propensity of neutrophils to reside at sites of inflammation, neutrophil-based assays and nanomedicine development have gained tremendous momentum in recent years [1,228–230]. 5-Hydroxytryptamine assembled onto nanoparticles containing photosensitizers and Zileuton (HZ-5 NPs), a novel therapeutic nanomedicine targeting tumor neutrophils' chemical and biological functions, can improve cancer therapy by providing sustained drug release. Leukotrienes released by neutrophils can promote the survival and translocation of circulating cancer cells in the lung and

eventually cause lung metastasis formation [71]. 5-Hydroxytryptamine (5-HT) was assembled onto NPs containing photosensitizers and zileuton (a leukotriene inhibitor) to obtain MPO and neutrophil-targeting HZ-5 NPs. Synthesized *bis*-5-HT has an MPO targeting function, where MPO is a neutrophil-specific enzyme [231]. HZ-5 NPs inhibit neutrophil-mediated lung metastasis through the sustained release of zileuton, thereby providing a novel strategy for improved lung cancer therapy and the exploration of a new nanomedicine drug design that exploits the TME [232] (Table 6).

Similarly, novel NPs for neutrophil detection have been extensively investigated. For example, protease-sensing NPs were used to monitor the dynamics of NE during lung infection and assess the efficacy of protease inhibitor therapy targeting NE in the treatment of α1 antitrypsin deficiency [233]. Near-infrared NPs can detect infiltrating neutrophil MPO activity [234]. Moreover, by combining human NE (HNE)-specific peptide substrates, quantum dots, and organic dyes, a fluorescence resonance energy transfer system capable of the *in vitro* detection and *in vivo* imaging of HNE was generated [235].

6.2. Nanotechnology targeting neutrophils in immunotherapy

Research into the use of NPs as cancer therapeutics has focused on their use as delivery platforms for conventional chemotherapeutic agents [26,236,237]. NP immunotherapy is among the least explored topics in this area and holds significant promise in oncology [25,238]. NPs can interact with and be taken up by innate immune cells to become immune-stimulatory agents that modulate the characteristics of natural immune cell populations [34,239]. A recent study found that inhaled empty self-assembled Cowpea Mosaic Virus (eCPMV) reduced lung metastasis from melanoma while producing potent systemic antitumor immunity against the primary tumor. The CPMV self-assembled into a 30-nm structure made up of 60 units 30 nm icosahedral structure consisting of 60 copies, each containing a different size of capsid protein unit. Inhaled eCPMV NPs were rapidly taken up by neutrophils in the TME, increasing the frequency of $CD11b^{+}Ly6G^{+}$-activated neutrophils and $CD11b^{+}CD86^{high}$ tumor-infiltrating neutrophils, which have potential antigen presentation and T cell priming ability. In this way, both the percentage (from 3% to 21%) and the total number of $CD45^{+}$ cells were significantly increased, inhibiting the tumor lung metastasis of melanoma by promoting regulatory T cell activation through modulating T cell receptor activation in response to antigen presentation. $CD11b^{+}CD86^{high}$ neutrophils can kill tumor cells by releasing reactive oxygen intermediates, priming $CD4^{+}$ T cells and inducing them toward a Th1 phenotype, and modulating NK cell function. $CD11b^{+}CD86^{high}$ neutrophils can produce CXCR3, CXCL9, and CXCL10, which recruit $CD4^{+}$ and $CD8^{+}$ T cells and generate an antitumor immune microenvironment [151]. In addition, NP-mediated NE inhibition may improve the efficacy of drugs that inhibit NET formation and thus reduce tumor metastasis [177].

Due to their inherent properties and drug-loading characteristics, NPs have become important tools in neutrophil detection and disease treatment. As research on NPs continues to intensify, we expect many new applications of NPs to be elucidated.

7. Conclusions and perspectives

Although multiple distinct populations of neutrophils have different functional characteristics, their lack of specific surface markers makes them challenging to distinguish. Neutrophils are highly plastic cell types that can respond rapidly to various stimuli in order to adapt to environmental changes, particularly in disease. Therefore, some researchers believe that neutrophil subpopulations may represent manifestations of neutrophil plasticity in response to disease stimulation rather than being naturally distinct populations. As research has progressed, discoveries have emerged that disprove traditional perceptions regarding neutrophils; for example, neutrophils have been classified into five (N1–5 or hN1–5) and six (mN1–6) subsets in humans and mice, respectively, rather than only being classified as N1 and N2 TANs [16]. Furthermore, CD36, CD41, CD61, and CD226 may be new markers that can differentiate LDNs from HDNs [113]; however, more evidence is needed to construct a new theoretical framework.

Neutrophils can promote tumor development through multiple pathways. After recruitment by the TME, neutrophils are stimulated by cytokines and chemokines, which alter the neutrophil properties. These altered neutrophils influence the recruitment and activation of inflammatory cells in the TME by releasing cytokines and enzymes. Subsequently, they create a beneficial immunosuppressive microenvironment for tumor development, metastasis, and angiogenesis, while playing an important role in the prognostic assessment of patients with tumors. Different types of neutrophils have varying functional mechanisms. PMN-MDSCs promote tumor progression primarily through immunosuppressive effects. In addition, they can induce T and NK cell incompetence, EMT, and MET, and can promote angiogenesis. TANs can promote tumor progression and inhibit antitumor immune responses through tumor suppressors, ROS, MMPs, NE, angiogenic elements, and numerous other factors [240–243]. In some cases, TANs can also be converted to an antitumor phenotype [244]. The antitumor activity of neutrophils is mainly attributable to the release of proinflammatory and immune-stimulatory molecules, such as IL-12 and TNF-α, or to TGF-β inhibition; these induce T cell recruitment and support tumor suppression. In addition, circulating $CD66b^{+}$ neutrophil populations can have immune-suppressive or proinflammatory functions in some acute and chronic inflammatory conditions and in the peripheral blood of patients with cancer, infections, or autoimmune diseases [98,103,245].

Nano-medicines, which represent a new treatment approach in the field of cancer, have been extensively studied due to their potential advantages over traditional treatments [230,246–249]. Nano-drug delivery systems can carry drugs to target sites and release them precisely to achieve better therapeutic effects and reduce drug side effects. Simultaneously, NP drug delivery systems can maintain stable blood levels in organisms by controlling the drug release rate. In recent decades, the rapid development of nanotechnology has led to the design and construction of nanoplatforms for various biomedical applications, including disease diagnosis and treatment [250–255]. Meanwhile, extensive research has demonstrated that NP-mediated delivery improves drug efficacy *in vivo*, decreases the clinical signs of lung injury, and reduces proinflammatory cytokines in the serum [256–259].

Tumor cells release chemokines, which attract neutrophils from the peripheral blood to the local TME, generating a local inflammatory environment that promotes tumor progression. Therefore, detecting neutrophils in tumors using nanotechnology and directly targeting neutrophils through nano-drug delivery systems to inhibit tumor progression have become hot research topics [232]. Many NPs have been used to detect neutrophil content and activity according to the NPs' properties [233–235,260]. For example, NE-based delivery vehicles can be primed with chemoattractants and migrate along a chemotactic gradient toward tumor cells infiltrating the inflamedtowards infiltrating tumor cells in the inflamed brain, then deliver drugs efficiently into tumor cells and induce cytotoxicity to inhibit tumor recurrence [194]. MPO has been widely used to develop molecular-based imaging probes that are retained at sites of inflammation by MPO-induced dimerization and protein attachment [261]. With continuous updating, we

believe that nanotechnology will become an indispensable tool for treating lung malignancies.

Acknowledgments

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Authors' contribution

Jian Zhang, Shasha Jiang, Shilin Li, Jipeng Jiang, Jie Mei, Yandong Chen, Yongfu Ma, Yang Liu, and Ying Liu contributed to writing this paper. All authors read and approved the final manuscript.

Compliance with ethics guidelines

Jian Zhang, Shasha Jiang, Shilin Li, Jipeng Jiang, Jie Mei, Yandong Chen, Yongfu Ma, Yang Liu, and Ying Liu declare that they have no conflict of interest or financial conflicts to disclose.

References

[1] Jiang J, Mei J, Yi S, Feng C, Ma Y, Liu Y, et al. Tumor associated macrophage and microbe: the potential targets of tumor vaccine delivery. Adv Drug Deliv Rev 2022;180:114046.
[2] Liu J, Feng X, Chen Z, Yang X, Shen Z, Guo M, et al. The adjuvant effect of $C_{60}(OH)_{22}$ nanoparticles promoting both humoral and cellular immune responses to HCV recombinant proteins. Mater Sci Eng C Mater Biol Appl 2019;97:753–9.
[3] Qiu N, Wang G, Wang J, Zhou Q, Guo M, Wang Y, et al. Tumor-associated macrophage and tumor-cell dually transfecting polyplexes for efficient interleukin-12 cancer gene therapy. Adv Mater 2021;33(2):e2006189.
[4] Tang J, Chen Z, Sun B, Dong J, Liu J, Zhou H, et al. Polyhydroxylated fullerenols regulate macrophage for cancer adoptive immunotherapy and greatly inhibit the tumor metastasis. Nanomedicine 2016;12(4):945–54.
[5] Oudkerk M, Liu S, Heuvelmans MA, Walter JE, Field JK. Lung cancer LDCT screening and mortality reduction—evidence, pitfalls and future perspectives. Nat Rev Clin Oncol 2021;18(3):135–51.
[6] Chen Z, Liu Y, Sun B, Li H, Dong J, Zhang L, et al. Polyhydroxylated metallofullerenols stimulate IL-1β secretion of macrophage through TLRs/MyD88/NF-κB pathway and $NLRP_3$ inflammasome activation. Small 2014;10(12):2362–72.
[7] Giron J, Lacout A, Marcy PY. Dealing with lung cancer TNM classification. J Thorac Oncol 2016;11(6):e77–8.
[8] Travis WD, Brambilla E, Noguchi M, Nicholson AG, Geisinger K, Yatabe Y, et al. Diagnosis of lung adenocarcinoma in resected specimens: implications of the 2011 International Association for the Study of Lung Cancer/American Thoracic Society/European Respiratory Society classification. Arch Pathol Lab Med 2013;137(5):685–705.
[9] Kirby T. Young non-smoker diagnosed with lung cancer. Lancet Respir Med 2020;8(2):141–2.
[10] Aulakh GK. Neutrophils in the lung: "the first responders". Cell Tissue Res 2018;371(3):577–88.
[11] Diem S, Schmid S, Krapf M, Flatz L, Born D, Jochum W, et al. Neutrophil-to-lymphocyte ratio (NLR) and platelet-to-lymphocyte ratio (PLR) as prognostic markers in patients with non-small cell lung cancer (NSCLC) treated with Nivolumab. Lung Cancer 2017;111:176–81.
[12] Mezquita L, Auclin E, Ferrara R, Charrier M, Remon J, Planchard D, et al. Association of the lung immune prognostic index with immune checkpoint inhibitor outcomes in patients with advanced non-small cell lung cancer. JAMA Oncol 2018;4(3):351–7.
[13] Kichenadasse G, Miners JO, Mangoni AA, Rowland A, Hopkins AM, Sorich MJ. Multiorgan immune-related adverse events during treatment with atezolizumab. J Natl Compr Canc Netw 2020;18(9):1191–9.
[14] Wisdom AJ, Hong CS, Lin AJ, Xiang Y, Cooper DE, Zhang J, et al. Neutrophils promote tumor resistance to radiation therapy. Proc Natl Acad Sci USA 2019;116(37):18584–9.
[15] Dinh HQ, Eggert T, Meyer MA, Zhu YP, Olingy CE, Llewellyn R, et al. Coexpression of CD71 and CD117 identifies an early unipotent neutrophil progenitor population in human bone marrow. Immunity 2020;53(2):319–34.e6.
[16] Zilionis R, Engblom C, Pfirschke C, Savova V, Zemmour D, Saatcioglu HD, et al. Single-cell transcriptomics of human and mouse lung cancers reveals conserved myeloid populations across individuals and species. Immunity 2019;50(5):1317–34.e10.
[17] Xiong S, Dong L, Cheng L. Neutrophils in cancer carcinogenesis and metastasis. J Hematol Oncol 2021;14(1):173.
[18] Liang W, Li Q, Ferrara N. Metastatic growth instructed by neutrophil-derived transferrin. Proc Natl Acad Sci USA 2018;115(43):11060–5.
[19] Sørensen OE, Borregaard N. Neutrophil extracellular traps—the dark side of neutrophils. J Clin Invest 2016;126(5):1612–20.
[20] Huang H, Zhang H, Onuma AE, Tsung A. Neutrophil elastase and neutrophil extracellular traps in the tumor microenvironment. Adv Experimental Medicine Biol 2020;1263:13–23.
[21] Jackaman C, Tomay F, Duong L, Abdol Razak NB, Pixley FJ, Metharom P, et al. Aging and cancer: the role of macrophages and neutrophils. Ageing Res Rev 2017;36:105–16.
[22] Wang Y, Cai R, Chen C. The nano–bio interactions of nanomedicines: understanding the biochemical driving forces and redox reactions. Acc Chem Res 2019;52(6):1507–18.
[23] Zhou H, Guo M, Li J, Qin F, Wang Y, Liu T, et al. Hypoxia-triggered self-assembly of ultrasmall iron oxide nanoparticles to amplify the imaging signal of a tumor. J Am Chem Soc 2021;143(4):1846–53.
[24] Davidson SM, Papagiannakopoulos T, Olenchock BA, Heyman JE, Keibler MA, Luengo A, et al. Environment impacts the metabolic dependencies of ras-driven non-small cell lung cancer. Cell Metab 2016;23(3):517–28.
[25] Li S, Xu S, Liang X, Xue Y, Mei J, Ma Y, et al. Nanotechnology: breaking the current treatment limits of lung cancer. Adv Healthc Mater 2021;10(12):e2100078.
[26] Liu Y, Chen C. Role of nanotechnology in HIV/AIDS vaccine development. Adv Drug Deliv Rev 2016;103:76–89.
[27] Liu Y, Chen C, Qian P, Lu X, Sun B, Zhang X, et al. Gd-metallofullerenol nanomaterial as non-toxic breast cancer stem cell-specific inhibitor. Nat Commun 2015;6:5988.
[28] Cao M, Cai R, Zhao L, Guo M, Wang L, Wang Y, et al. Molybdenum derived from nanomaterials incorporates into molybdenum enzymes and affects their activities in vivo. Nat Nanotechnol 2021;16(6):708–16.
[29] Lu X, Zhu Y, Bai R, Wu Z, Qian W, Yang L, et al. Long-term pulmonary exposure to multi-walled carbon nanotubes promotes breast cancer metastatic cascades. Nat Nanotechnol 2019;14(7):719–27.
[30] Hosseinalizadeh H, Mahmoodpour M, Razaghi Bahabadi Z, Hamblin MR, Mirzaei H. Neutrophil mediated drug delivery for targeted glioblastoma therapy: a comprehensive review. Biomed Pharmacother 2022;156:113841.
[31] Chen Y, Hu H, Tan S, Dong Q, Fan X, Wang Y, et al. The role of neutrophil extracellular traps in cancer progression, metastasis and therapy. Exp Hematol Oncol 2022;11(1):99.
[32] Meng H, Leong W, Leong KW, Chen C, Zhao Y. Walking the line: the fate of nanomaterials at biological barriers. Biomaterials 2018;174:41–53.
[33] Wang X, Wang M, Lei R, Zhu SF, Zhao Y, Chen C. Chiral surface of nanoparticles determines the orientation of adsorbed transferrin and its interaction with receptors. ACS Nano 2017;11(5):4606–16.
[34] Lu X, Zhu T, Chen C, Liu Y. Right or left: the role of nanoparticles in pulmonary diseases. Int J Mol Sci 2014;15(10):17577–600.
[35] Pan J, Xue Y, Li S, Wang L, Mei J, Ni D, et al. $PM_{2.5}$ induces the distant metastasis of lung adenocarcinoma via promoting the stem cell properties of cancer cells. Environ Pollut 2022;296:118718.
[36] Wu P, Yin D, Liu J, Zhou H, Guo M, Liu J, et al. Cell membrane based biomimetic nanocomposites for targeted therapy of drug resistant *EGFR*-mutated lung cancer. Nanoscale 2019;11(41):19520–8.
[37] Li Y, Liu Y, Fu Y, Wei T, Le Guyader L, Gao G, et al. The triggering of apoptosis in macrophages by pristine graphene through the MAPK and TGF-β signaling pathways. Biomaterials 2012;33(2):402–11.
[38] Liu Y, Jiao F, Qiu Y, Li W, Lao F, Zhou G, et al. The effect of $Gd@C_{82}(OH)_{22}$ nanoparticles on the release of Th1/Th2 cytokines and induction of TNF-α mediated cellular immunity. Biomaterials 2009;30(23–24):3934–45.
[39] Liu Y, Jiao F, Qiu Y, Li W, Qu Y, Tian C, et al. Immunostimulatory properties and enhanced TNF-α mediated cellular immunity for tumor therapy by $C_{60}(OH)_{20}$ nanoparticles. Nanotechnology 2009;20(41):415102.
[40] Kang MH, Go SI, Song HN, Lee A, Kim SH, Kang JH, et al. The prognostic impact of the neutrophil-to-lymphocyte ratio in patients with small-cell lung cancer. Br J Cancer 2014;111(3):452–60.
[41] Yee J, Sadar MD, Sin DD, Kuzyk M, Xing L, Kondra J, et al. Connective tissue-activating peptide III: a novel blood biomarker for early lung cancer detection. J Clin Oncol 2009;27(17):2787–92.
[42] Duruisseaux M, Martínez-Cardús A, Calleja-Cervantes ME, Moran S, Castro de Moura M, Davalos V, et al. Epigenetic prediction of response to anti-PD-1 treatment in non-small-cell lung cancer: a multicentre, retrospective analysis. Lancet. Respir Med 2018;6(10):771–81.
[43] Kadota K, Nitadori JI, Ujiie H, Buitrago DH, Woo KM, Sima CS, et al. Prognostic impact of immune microenvironment in lung squamous cell carcinoma: tumor-infiltrating $CD10^+$ neutrophil/$CD20^+$ lymphocyte ratio as an independent prognostic factor. J Thorac Oncol 2015;10(9):1301–10.
[44] Xie D, Marks R, Zhang M, Jiang G, Jatoi A, Garces YI, et al. Nomograms predict overall survival for patients with small-cell lung cancer incorporating pretreatment peripheral blood markers. J Thorac Oncol 2015;10(8):1213–20.

[45] Shaul ME, Fridlender ZG. Tumour-associated neutrophils in patients with cancer. Nat Rev Clin Oncol 2019;16(10):601–20.
[46] Cannon NA, Meyer J, Iyengar P, Ahn C, Westover KD, Choy H, et al. Neutrophil–lymphocyte and platelet–lymphocyte ratios as prognostic factors after stereotactic radiation therapy for early-stage non-small-cell lung cancer. J Thorac Oncol 2015;10(2):280–5.
[47] Hopkins AM, Kichenadasse G, Garrett-Mayer E, Karapetis CS, Rowland A, Sorich MJ. Development and validation of a prognostic model for patients with advanced lung cancer treated with the immune checkpoint inhibitor atezolizumab. Clin Cancer Res 2020;26(13):3280–6.
[48] Jiang M, Peng W, Pu X, Chen B, Li J, Xu F, et al. Peripheral blood biomarkers associated with outcome in non-small cell lung cancer patients treated with nivolumab and durvalumab monotherapy. Front Oncol 2020;10:913.
[49] Kazandjian D, Gong Y, Keegan P, Pazdur R, Blumenthal GM. Prognostic value of the lung immune prognostic index for patients treated for metastatic non-small cell lung cancer. JAMA Oncol 2019;5(10):1481–5.
[50] Yao Y, Yuan D, Liu H, Gu X, Song Y. Pretreatment neutrophil to lymphocyte ratio is associated with response to therapy and prognosis of advanced non-small cell lung cancer patients treated with first-line platinum-based chemotherapy. Cancer Immunol Immunother 2013;62(3):471–9.
[51] Sun B, Brooks ED, Komaki R, Liao Z, Jeter M, McAleer M, et al. Long-term outcomes of salvage stereotactic ablative radiotherapy for isolated lung recurrence of non-small cell lung cancer: a phase II clinical trial. J Thorac Oncol 2017;12(6):983–92.
[52] Kim Y, Kim CH, Lee HY, Lee SH, Kim HS, Lee S, et al. Comprehensive clinical and genetic characterization of hyperprogression based on volumetry in advanced non-small cell lung cancer treated with immune checkpoint inhibitor. J Thorac Oncol 2019;14(9):1608–18.
[53] Castello A, Rossi S, Mazziotti E, Toschi L, Lopci E. Hyperprogressive disease in patients with non-small cell lung cancer treated with checkpoint inhibitors: the role of ^{18}F-FDG PET/CT. J Nucl Med 2020;61(6):821–6.
[54] Teramukai S, Kitano T, Kishida Y, Kawahara M, Kubota K, Komuta K, et al. Pretreatment neutrophil count as an independent prognostic factor in advanced non-small-cell lung cancer: an analysis of Japan Multinational Trial Organisation LC00-03. Eur J Cancer 2009;45(11):1950–8.
[55] Li B, Cui Y, Diehn M, Li R. Development and validation of an individualized immune prognostic signature in early-stage nonsquamous non-small cell lung cancer. JAMA Oncol 2017;3(11):1529–37.
[56] Pore N, Wu S, Standifer N, Jure-Kunkel M, de Los RM, Shrestha Y, et al. Resistance to durvalumab and durvalumab plus tremelimumab is associated with functional *STK11* mutations in patients with non-small cell lung cancer and is reversed by *STAT3* knockdown. Cancer Discov 2021;11(11):2828–45.
[57] Tanizaki J, Haratani K, Hayashi H, Chiba Y, Nakamura Y, Yonesaka K, et al. Peripheral blood biomarkers associated with clinical outcome in non-small cell lung cancer patients treated with Nivolumab. J Thorac Oncol 2018;13(1):97–105.
[58] Cowland JB, Borregaard N. Granulopoiesis and granules of human neutrophils. Immunol Rev 2016;273(1):11–28.
[59] Galli SJ, Borregaard N, Wynn TA. Phenotypic and functional plasticity of cells of innate immunity: macrophages, mast cells and neutrophils. Nat Immunol 2011;12(11):1035–44.
[60] Lawrence SM, Corriden R, Nizet V. The ontogeny of a neutrophil: mechanisms of granulopoiesis and homeostasis. Microbiol Mol Biol Rev 2018;82(1):e00057–117.
[61] Yin C, Heit B. Armed for destruction: formation, function and trafficking of neutrophil granules. Cell Tissue Res 2018;371(3):455–71.
[62] Othman A, Sekheri M, Filep JG. Roles of neutrophil granule proteins in orchestrating inflammation and immunity. FEBS J 2022;289(14):3932–53.
[63] Rørvig S, Østergaard O, Heegaard NH, Borregaard N. Proteome profiling of human neutrophil granule subsets, secretory vesicles, and cell membrane: correlation with transcriptome profiling of neutrophil precursors. J Leukoc Biol 2013;94(4):711–21.
[64] Vols S, Sionov RV, Granot Z. Always look on the bright side: anti-tumor functions of neutrophils. Curr Pharm Des 2017;23(32):4862–92.
[65] Antonio N, Bønnelykke-Behrndtz ML, Ward LC, Collin J, Christensen IJ, Steiniche T, et al. The wound inflammatory response exacerbates growth of pre-neoplastic cells and progression to cancer. EMBO J 2015;34(17):2219–36.
[66] García-Mendoza MG, Inman DR, Ponik SM, Jeffery JJ, Sheerar DS, van Doorn RR, et al. Neutrophils drive accelerated tumor progression in the collagen-dense mammary tumor microenvironment. Breast Cancer Res 2016;18(1):49.
[67] Janiszewska M, Tabassum DP, Castaño Z, Cristea S, Yamamoto KN, Kingston NL, et al. Subclonal cooperation drives metastasis by modulating local and systemic immune microenvironments. Nat Cell Biol 2019;21(7):879–88.
[68] Khou S, Popa A, Luci C, Bihl F, Meghraoui-Kheddar A, Bourdely P, et al. Tumor-associated neutrophils dampen adaptive immunity and promote cutaneous squamous cell carcinoma development. Cancers 2020;12(7):1860.
[69] Jablonska J, Leschner S, Westphal K, Lienenklaus S, Weiss S. Neutrophils responsive to endogenous IFN-β regulate tumor angiogenesis and growth in a mouse tumor model. J Clin Invest 2010;120(4):1151–64.
[70] Spicer JD, McDonald B, Cools-Lartigue JJ, Chow SC, Giannias B, Kubes P, et al. Neutrophils promote liver metastasis via Mac-1-mediated interactions with circulating tumor cells. Cancer Res 2012;72(16):3919–27.
[71] Wculek SK, Malanchi I. Neutrophils support lung colonization of metastasis-initiating breast cancer cells. Nature 2015;528(7582):413–7.
[72] Tyagi A, Sharma S, Wu K, Wu SY, Xing F, Liu Y, et al. Nicotine promotes breast cancer metastasis by stimulating N_2 neutrophils and generating pre-metastatic niche in lung. Nat Commun 2021;12(1):474.
[73] Xiao Y, Cong M, Li J, He D, Wu Q, Tian P, et al. Cathepsin C promotes breast cancer lung metastasis by modulating neutrophil infiltration and neutrophil extracellular trap formation. Cancer Cell 2021;39(3):423–37.e7.
[74] El Rayes T, Catena R, Lee S, Stawowczyk M, Joshi N, Fischbach C, et al. Lung inflammation promotes metastasis through neutrophil protease-mediated degradation of TSP-1. Proc Natl Acad Sci USA 2015;112(52):16000–5.
[75] Faget J, Groeneveld S, Boivin G, Sankar M, Zangger N, Garcia M, et al. Neutrophils and snail orchestrate the establishment of a pro-tumor microenvironment in lung cancer. Cell Rep 2017;21(11):3190–204.
[76] Jamieson T, Clarke M, Steele CW, Samuel MS, Neumann J, Jung A, et al. Inhibition of CXCR2 profoundly suppresses inflammation-driven and spontaneous tumorigenesis. J Clin Invest 2012;122(9):3127–44.
[77] Kowanetz M, Wu X, Lee J, Tan M, Hagenbeek T, Qu X, et al. Granulocyte-colony stimulating factor promotes lung metastasis through mobilization of Ly6G$^+$Ly6C$^+$ granulocytes. Proc Natl Acad Sci USA 2010;107(50):21248–55.
[78] Gordon-Weeks AN, Lim SY, Yuzhalin AE, Jones K, Markelc B, Kim KJ, et al. Neutrophils promote hepatic metastasis growth through fibroblast growth factor 2-dependent angiogenesis in mice. Hepatology 2017;65(6):1920–35.
[79] Coffelt SB, Kersten K, Doornebal CW, Weiden J, Vrijland K, Hau CS, et al. IL-17-producing γδ T cells and neutrophils conspire to promote breast cancer metastasis. Nature 2015;522(7556):345–8.
[80] Tabariès S, Ouellet V, Hsu BE, Annis MG, Rose AA, Meunier L, et al. Granulocytic immune infiltrates are essential for the efficient formation of breast cancer liver metastases. Breast Cancer Res 2015;17(1):45.
[81] Forsthuber A, Lipp K, Andersen L, Ebersberger S, Graña-Castro EW, et al. CXCL5 as regulator of neutrophil function in cutaneous melanoma. J Invest Dermatol 2019;139(1):186–94.
[82] Granot Z, Henke E, Comen EA, King TA, Norton L, Benezra R. Tumor entrained neutrophils inhibit seeding in the premetastatic lung. Cancer Cell 2011;20(3):300–14.
[83] López-Lago MA, Posner S, Thodima VJ, Molina AM, Motzer RJ, Chaganti RS. Neutrophil chemokines secreted by tumor cells mount a lung antimetastatic response during renal cell carcinoma progression. Oncogene 2013;32(14):1752–60.
[84] Fridlender ZG, Sun J, Kim S, Kapoor V, Cheng G, Ling L, et al. Polarization of tumor-associated neutrophil phenotype by TGF-β: "N1" versus "N2" TAN. Cancer Cell 2009;16(3):183–94.
[85] Gershkovitz M, Caspi Y, Fainsod-Levi T, Katz B, Michaeli J, Khawaled S, et al. TRPM2 mediates neutrophil killing of disseminated tumor cells. Cancer Res 2018;78(10):2680–90.
[86] Sanford MA, Yan Y, Canfield SE, Hassan W, Selleck WA, Atkinson G, et al. Independent contributions of GR-1$^+$ leukocytes and Fas/FasL interactions to induce apoptosis following interleukin-12 gene therapy in a metastatic model of prostate cancer. Hum Gene Ther 2001;12(12):1485–98.
[87] Schaider H, Oka M, Bogenrieder T, Nesbit M, Satyamoorthy K, Berking C, et al. Differential response of primary and metastatic melanomas to neutrophils attracted by IL-8. Int J Cancer 2003;103(3):335–43.
[88] Li P, Lu M, Shi J, Hua L, Gong Z, Li Q, et al. Dual roles of neutrophils in metastatic colonization are governed by the host NK cell status. Nat Commun 2020;11(1):4387.
[89] Läubli H, Pearce OM, Schwarz F, Siddiqui SS, Deng L, Stanczak MA, et al. Engagement of myelomonocytic Siglecs by tumor-associated ligands modulates the innate immune response to cancer. Proc Natl Acad Sci USA 2014;111(39):14211–6.
[90] Uyanik B, Goloudina AR, Akbarali A, Grigorash BB, Petukhov AV, Singhal S, et al. Inhibition of the DNA damage response phosphatase PPM1D reprograms neutrophils to enhance anti-tumor immune responses. Nat Commun 2021;12(1):3622.
[91] Liu Y, O'Leary CE, Wang LS, Bhatti TR, Dai N, Kapoor V, et al. CD11b$^+$Ly6G$^+$ cells inhibit tumor growth by suppressing IL-17 production at early stages of tumorigenesis. Oncoimmunology 2015;5(1):e1061175.
[92] Hagerling C, Gonzalez H, Salari K, Wang CY, Lin C, Robles I, et al. Immune effector monocyte-neutrophil cooperation induced by the primary tumor prevents metastatic progression of breast cancer. Proc Natl Acad Sci USA 2019;116(43):21704–14.
[93] Desbois M, Béal C, Charrier M, Besse B, Meurice G, Cagnard N, et al. IL-15 superagonist RLI has potent immunostimulatory properties on NK cells: implications for antimetastatic treatment. J Immunother Cancer 2020;8(1):e000632.
[94] Singhal S, Bhojnagarwala PS, O'Brien S, Moon EK, Garfall AL, Rao AS, et al. Origin and role of a subset of tumor-associated neutrophils with antigen-presenting cell features in early-stage human lung cancer. Cancer Cell 2016;30(1):120–35.
[95] Costanzo-Garvey DL, Keeley T, Case AJ, Watson GF, Alsamraae M, Yu Y, et al. Neutrophils are mediators of metastatic prostate cancer progression in bone. Cancer Immunol Immunother 2020;69(6):1113–30.
[96] Raftopoulou S, Valadez-Cosmes P, Mihalic ZN, Schicho R, Kargl J. Tumor-mediated neutrophil polarization and therapeutic implications. Int J Mol Sci 2022;23(6):3218.
[97] Sionov RV. Leveling up the controversial role of neutrophils in cancer: when the complexity becomes entangled. Cells 2021;10(9):2486.
[98] Dumitru CA, Moses K, Trellakis S, Lang S, Brandau S. Neutrophils and granulocytic myeloid-derived suppressor cells: immunophenotyping, cell

biology and clinical relevance in human oncology. Cancer Immunol Immunother 2012;61(8):1155–67.
[99] Wu F, Fan J, He Y, Xiong A, Yu J, Li Y, et al. Single-cell profiling of tumor heterogeneity and the microenvironment in advanced non-small cell lung cancer. Nat Commun 2021;12(1):2540.
[100] Shaul ME, Levy L, Sun J, Mishalian I, Singhal S, Kapoor V, et al. Tumor-associated neutrophils display a distinct N1 profile following TGF-β modulation: a transcriptomics analysis of pro- vs. antitumor TANs. Oncoimmunology 2016;5(11):e1232221.
[101] Mishalian I, Bayuh R, Levy L, Zolotarov L, Michaeli J, Fridlender ZG. Tumor-associated neutrophils (TAN) develop pro-tumorigenic properties during tumor progression. Cancer Immunol Immunother 2013;62(11):1745–56.
[102] Piccard H, Muschel RJ, Opdenakker G. On the dual roles and polarized phenotypes of neutrophils in tumor development and progression. Crit Rev Oncol Hematol 2012;82(3):296–309.
[103] Carmona-Rivera C, Kaplan MJ. Low-density granulocytes: a distinct class of neutrophils in systemic autoimmunity. Semin Immunopathol 2013;35(4):455–63.
[104] Fu J, Tobin MC, Thomas LL. Neutrophil-like low-density granulocytes are elevated in patients with moderate to severe persistent asthma. Ann Allergy Asthma Immunol 2014;113(6):635–40.e2.
[105] Sagiv JY, Voels S, Granot Z. Isolation and characterization of low- vs. high-density neutrophils in cancer. Methods Mol Biol 2016;1458:179–93.
[106] Grecian R, Whyte MKB, Walmsley SR. The role of neutrophils in cancer. Br Med Bull 2018;128(1):5–14.
[107] Shaul ME, Eyal O, Guglietta S, Aloni P, Zlotnik A, Forkosh E, et al. Circulating neutrophil subsets in advanced lung cancer patients exhibit unique immune signature and relate to prognosis. FASEB J 2020;34(3):4204–18.
[108] Ui Mhaonaigh A, Coughlan AM, Dwivedi A, Hartnett J, Cabral J, Moran B, et al. Low density granulocytes in ANCA vasculitis are heterogenous and hypo-responsive to anti-myeloperoxidase antibodies. Front Immunol 2019;10:2603.
[109] Hassani M, Hellebrekers P, Chen N, van Aalst C, Bongers S, Hietbrink F, et al. On the origin of low-density neutrophils. J Leukoc Biol 2020;107(5):809–18.
[110] Pitt JM, Marabelle A, Eggermont A, Soria JC, Kroemer G, Zitvogel L. Targeting the tumor microenvironment: removing obstruction to anticancer immune responses and immunotherapy. Ann Oncol 2016;27(8):1482–92.
[111] Remark R, Becker C, Gomez JE, Damotte D, Dieu-Nosjean MC, Sautès-Fridman C, et al. The non-small cell lung cancer immune contexture. A major determinant of tumor characteristics and patient outcome. Am J Respir Crit Care Med 2015;191(4):377–90.
[112] Tay SH, Celhar T, Fairhurst AM. Low-density neutrophils in systemic lupus erythematosus. Arthritis Rheumatol 2020;72(10):1587–95.
[113] Valadez-Cosmes P, Maitz K, Kindler O, Raftopoulou S, Kienzl M, Santiso A, et al. Identification of novel low-density neutrophil markers through unbiased high-dimensional flow cytometry screening in non-small cell lung cancer patients. Front Immunol 2021;12:703846.
[114] Condamine T, Mastio J, Gabrilovich DI. Transcriptional regulation of myeloid-derived suppressor cells. J Leukoc Biol 2015;98(6):913–22.
[115] Dysthe M, Parihar R. Myeloid-derived suppressor cells in the tumor microenvironment. Adv Experimental Med Biol 2020;1224:117–40.
[116] Gabrilovich DI. Myeloid-derived suppressor cells. Cancer Immunol Res 2017;5(1):3–8.
[117] Grover A, Sanseviero E, Timosenko E, Gabrilovich DI. Myeloid-derived suppressor cells: a propitious road to clinic. Cancer Discov 2021;11(11):2693–706.
[118] Kumar V, Patel S, Tcyganov E, Gabrilovich DI. The nature of myeloid-derived suppressor cells in the tumor microenvironment. Trends Immunol 2016;37(3):208–20.
[119] Law AMK, Valdes-Mora F, Gallego-Ortega D. Myeloid-derived suppressor cells as a therapeutic target for cancer. Cells 2020;9(3):561.
[120] Movahedi K, Guilliams M, Van den Bossche J, Van den Bergh R, Gysemans C, Beschin A, et al. Identification of discrete tumor-induced myeloid-derived suppressor cell subpopulations with distinct T cell-suppressive activity. Blood 2008;111(8):4233–44.
[121] Ostrand-Rosenberg S, Sinha P. Myeloid-derived suppressor cells: linking inflammation and cancer. J Immunol 2009;182(8):4499–506.
[122] Pillay J, Tak T, Kamp VM, Koenderman L. Immune suppression by neutrophils and granulocytic myeloid-derived suppressor cells: similarities and differences. Cell Mol Life Sci 2013;70(20):3813–27.
[123] Veglia F, Hashimoto A, Dweep H, Sanseviero E, De Leo A, Tcyganov E, et al. Analysis of classical neutrophils and polymorphonuclear myeloid-derived suppressor cells in cancer patients and tumor-bearing mice. J Exp Med 2021;218(4):e20201803.
[124] Barrera L, Montes-Servín E, Hernandez-Martinez JM, Orozco-Morales M, Montes-Servín E, Michel-Tello D, et al. Levels of peripheral blood polymorphonuclear myeloid-derived suppressor cells and selected cytokines are potentially prognostic of disease progression for patients with non-small cell lung cancer. Cancer Immunol Immunother 2018;67(9):1393–406.
[125] Solito S, Marigo I, Pinton L, Damuzzo V, Mandruzzato S, Bronte V. Myeloid-derived suppressor cell heterogeneity in human cancers. Ann N Y Acad Sci 2014;1319:47–65.
[126] Talmadge JE, Gabrilovich DI. History of myeloid-derived suppressor cells. Nat Rev Cancer 2013;13(10):739–52.
[127] Marini O, Costa S, Bevilacqua D, Calzetti F, Tamassia N, Spina C, et al. Mature $CD10^{+}$ and immature $CD10^{-}$ neutrophils present in G-CSF-treated donors display opposite effects on T cells. Blood 2017;129(10):1343–56.
[128] Peng Z, Liu C, Victor AR, Cao DY, Veiras LC, Bernstein EA, et al. Tumors exploit $CXCR4^{hi}CD62L^{lo}$ aged neutrophils to facilitate metastatic spread. Oncoimmunology 2021;10(1):1870811.
[129] Yang C, Wang Z, Li L, Zhang Z, Jin X, Wu P, et al. Aged neutrophils form mitochondria-dependent vital NETs to promote breast cancer lung metastasis. J Immunother Cancer 2021;9(10):e002875.
[130] Millrud CR, Kågedal Å, Kumlien Georén S, Winqvist O, Uddman R, Razavi R, et al. NET-producing $CD16^{high}$ $CD62L^{dim}$ neutrophils migrate to tumor sites and predict improved survival in patients with HNSCC. Int J Cancer 2017;140(11):2557–67.
[131] Schroeter A, Roesel MJ, Matsunaga T, Xiao Y, Zhou H, Tullius SG. Aging affects the role of myeloid-derived suppressor cells in alloimmunity. Front Immunol 2022;13:917972.
[132] Tcyganov E, Mastio J, Chen E, Gabrilovich DI. Plasticity of myeloid-derived suppressor cells in cancer. Curr Opin Immunol 2018;51:76–82.
[133] Hegde S, Leader AM, Merad M. MDSC: markers, development, states, and unaddressed complexity. Immunity 2021;54(5):875–84.
[134] Rosales C. Neutrophil: a cell with many roles in inflammation or several cell types? Front Physiol 2018;9:113.
[135] Favaloro J, Liyadipitiya T, Brown R, Yang S, Suen H, Woodland N, et al. Myeloid derived suppressor cells are numerically, functionally and phenotypically different in patients with multiple myeloma. Leuk Lymphoma 2014;55(12):2893–900.
[136] Cassetta L, Baekkevold ES, Brandau S, Bujko A, Cassatella MA, Dorhoi A, et al. Deciphering myeloid-derived suppressor cells: isolation and markers in humans, mice and non-human primates. Cancer Immunol Immunother 2019;68(4):687–97.
[137] Kusmartsev SA, Li Y, Chen SH. $Gr\text{-}1^{+}$ myeloid cells derived from tumor-bearing mice inhibit primary T cell activation induced through CD3/CD28 costimulation. J Immunol 2000;165(2):779–85.
[138] Keskinov AA, Shurin MR. Myeloid regulatory cells in tumor spreading and metastasis. Immunobiology 2015;220(2):236–42.
[139] Blaisdell A, Crequer A, Columbus D, Daikoku T, Mittal K, Dey SK, et al. Neutrophils oppose uterine epithelial carcinogenesis via debridement of hypoxic tumor cells. Cancer Cell 2015;28(6):785–99.
[140] Sionov RV, Fridlender ZG, Granot Z. The multifaceted roles neutrophils play in the tumor microenvironment. Cancer Microenviron 2015;8(3):125–58.
[141] Andzinski L, Kasnitz N, Stahnke S, Wu CF, Gereke M, von Köckritz-Blickwede M, et al. Type I IFNs induce anti-tumor polarization of tumor associated neutrophils in mice and human. Int J Cancer 2016;138(8):1982–93.
[142] Yan B, Wei JJ, Yuan Y, Sun R, Li D, Luo J, et al. IL-6 cooperates with G-CSF to induce protumor function of neutrophils in bone marrow by enhancing STAT3 activation. J Immunol 2013;190(11):5882–93.
[143] Spiegel A, Brooks MW, Houshyar S, Reinhardt F, Ardolino M, Fessler E, et al. Neutrophils suppress intraluminal NK cell-mediated tumor cell clearance and enhance extravasation of disseminated carcinoma cells. Cancer Discov 2016;6(6):630–49.
[144] Hadjigol S, Shah BA, O'Brien-Simpson NM. The 'danse macabre'—neutrophils the interactive partner affecting oral cancer outcomes. Front Immunol 2022;13:894021.
[145] Mishalian I, Bayuh R, Eruslanov E, Michaeli J, Levy L, Zolotarov L, et al. Neutrophils recruit regulatory T-cells into tumors via secretion of CCL17–a new mechanism of impaired antitumor immunity. Int J Cancer 2014;135(5):1178–86.
[146] Zou JM, Qin J, Li YC, Wang Y, Li D, Shu Y, et al. IL-35 induces N2 phenotype of neutrophils to promote tumor growth. Oncotarget 2017;8(20):33501–14.
[147] Schernberg A, Blanchard P, Chargari C, Deutsch E. Neutrophils, a candidate biomarker and target for radiation therapy? Acta Oncol 2017;56(11):1522–30.
[148] Jungnickel C, Schmidt LH, Bittigkoffer L, Wolf L, Wolf A, Ritzmann F, et al. IL-17C mediates the recruitment of tumor-associated neutrophils and lung tumor growth. Oncogene 2017;36(29):4182–90.
[149] Tie Y, Tang F, Wei YQ, Wei XW. Immunosuppressive cells in cancer: mechanisms and potential therapeutic targets. J Hematol Oncol 2022;15(1):61.
[150] Yu X, Liu W, Chen S, Cheng X, Paez PA, Sun T, et al. Immunologically programming the tumor microenvironment induces the pattern recognition receptor NLRC4-dependent antitumor immunity. J Immunother Cancer 2021;9(1):e001595.
[151] Lizotte PH, Wen AM, Sheen MR, Fields J, Rojanasopondist P, Steinmetz NF, et al. In situ vaccination with cowpea mosaic virus nanoparticles suppresses metastatic cancer. Nat Nanotechnol 2016;11(3):295–303.
[152] Davis RE, Sharma S, Conceição J, Carneiro P, Novais F, Scott P, et al. Phenotypic and functional characteristics of $HLA\text{-}DR^{+}$ neutrophils in Brazilians with cutaneous leishmaniasis. J Leukoc Biol 2017;101(3):739–49.
[153] Galdiero MR, Varricchi G, Loffredo S, Mantovani A, Marone G. Roles of neutrophils in cancer growth and progression. J Leukoc Biol 2018;103(3):457–64.
[154] Sagiv JY, Michaeli J, Assi S, Mishalian I, Kisos H, Levy L, et al. Phenotypic diversity and plasticity in circulating neutrophil subpopulations in cancer. Cell Rep 2015;10(4):562–73.

[155] Kargl J, Busch SE, Yang GH, Kim KH, Hanke ML, Metz HE, et al. Neutrophils dominate the immune cell composition in non-small cell lung cancer. Nat Commun 2017;8:14381.
[156] Rakoff-Nahoum S. Why cancer and inflammation? Yale J Biol Med 2006;79 (3–4):123–30.
[157] Rojas A, GonzÁlez I, Araya P. RAGE in cancer lung: the end of a long and winding road is in sight. Chin J Lung Cancer 2018;21(9):655–7. Chinese.
[158] Coussens LM, Tinkle CL, Hanahan D, Werb Z. MMP-9 supplied by bone marrow-derived cells contributes to skin carcinogenesis. Cell 2000;103 (3):481–90.
[159] Bergers G, Brekken R, McMahon G, Vu TH, Itoh T, Tamaki K, et al. Matrix metalloproteinase-9 triggers the angiogenic switch during carcinogenesis. Nat Cell Biol 2000;2(10):737–44.
[160] Liao D, Liu Z, Wrasidlo WJ, Luo Y, Nguyen G, Chen T, et al. Targeted therapeutic remodeling of the tumor microenvironment improves an HER-2 DNA vaccine and prevents recurrence in a murine breast cancer model. Cancer Res 2011;71(17):5688–96.
[161] De Palma M, Biziato D, Petrova TV. Microenvironmental regulation of tumour angiogenesis. Nat Rev Cancer 2017;17(8):457–74.
[162] Acuff HB, Carter KJ, Fingleton B, Gorden DL, Matrisian LM. Matrix metalloproteinase-9 from bone marrow-derived cells contributes to survival but not growth of tumor cells in the lung microenvironment. Cancer Res 2006;66(1):259–66.
[163] Taniguchi K, Yang P, Jett J, Bass E, Meyer R, Wang Y, et al. Polymorphisms in the promoter region of the neutrophil elastase gene are associated with lung cancer development. Clin Cancer Res 2002;8(4):1115–20.
[164] Houghton AM, Rzymkiewicz DM, Ji H, Gregory AD, Egea EE, Metz HE, et al. Neutrophil elastase-mediated degradation of IRS-1 accelerates lung tumor growth. Nat Med 2010;16(2):219–23.
[165] Saleh AD, Cheng H, Martin SE, Si H, Ormanoglu P, Carlson S, et al. Integrated genomic and functional microRNA analysis identifies miR-30-5p as a tumor suppressor and potential therapeutic nanomedicine in head and neck cancer. Clin Cancer Res 2019;25(9):2860–73.
[166] Verma NK, Dempsey E, Long A, Davies A, Barry SP, Fallon PG, et al. Leukocyte function-associated antigen-1/intercellular adhesion molecule-1 interaction induces a novel genetic signature resulting in T-cells refractory to transforming growth factor-β signaling. J Biol Chem 2012;287(32):27204–16.
[167] Wislez M, Antoine M, Rabbe N, Gounant V, Poulot V, Lavolé A, et al. Neutrophils promote aerogenous spread of lung adenocarcinoma with bronchioloalveolar carcinoma features. Clin Cancer Res 2007;13(12): 3518–27.
[168] Wislez M, Rabbe N, Marchal J, Milleron B, Crestani B, Mayaud C, et al. Hepatocyte growth factor production by neutrophils infiltrating bronchioloalveolar subtype pulmonary adenocarcinoma: role in tumor progression and death. Cancer Res 2003;63(6):1405–12.
[169] Stewart PA, Welsh EA, Slebos RJC, Fang B, Izumi V, Chambers M, et al. Proteogenomic landscape of squamous cell lung cancer. Nat Commun 2019;10(1):3578.
[170] Shen J, Hao J, Chen Y, Liu H, Wu J, Hu B, et al. Neutrophil-mediated clinical nanodrug for treatment of residual tumor after focused ultrasound ablation. J Nanobiotechnology 2021;19(1):345.
[171] Akbay EA, Koyama S, Liu Y, Dries R, Bufe LE, Silkes M, et al. Interleukin-17A promotes lung tumor progression through neutrophil attraction to tumor sites and mediating resistance to PD-1 blockade. J Thorac Oncol 2017;12 (8):1268–79.
[172] Gillette MA, Satpathy S, Cao S, Dhanasekaran SM, Vasaikar SV, Krug K, et al.; CPTAC. Proteogenomic characterization reveals therapeutic vulnerabilities in lung adenocarcinoma. Cell 2020;182(1):200–25.e35.
[173] Perego M, Tyurin VA, Tyurina YY, Yellets J, Nacarelli T, Lin C, et al. Reactivation of dormant tumor cells by modified lipids derived from stress-activated neutrophils. Sci Transl Med 2020;12(572). eabb5817.
[174] Chen J, Hou S, Liang Q, He W, Li R, Wang H, et al. Localized degradation of neutrophil extracellular traps by photoregulated enzyme delivery for cancer immunotherapy and metastasis suppression. ACS Nano 2022;16(2): 2585–97.
[175] Albrengues J, Shields MA, Ng D, Park CG, Ambrico A, Poindexter ME, et al. Neutrophil extracellular traps produced during inflammation awaken dormant cancer cells in mice. Science 2018;361(6409):eaao4227.
[176] Zeiher BG, Artigas A, Vincent JL, Dmitrienko A, Jackson K, Thompson BT, et al. STRIVE Study Group. Neutrophil elastase inhibition in acute lung injury: results of the STRIVE study. Crit Care Med 2004;32(8):1695–702.
[177] Okeke EB, Louttit C, Fry C, Najafabadi AH, Han K, Nemzek J, et al. Inhibition of neutrophil elastase prevents neutrophil extracellular trap formation and rescues mice from endotoxic shock. Biomaterials 2020;238:119836.
[178] Gershkovitz M, Fainsod-Levi T, Khawaled S, Shaul ME, Sionov RV, Cohen-Daniel L, et al. Microenvironmental cues determine tumor cell susceptibility to neutrophil cytotoxicity. Cancer Res 2018;78(17):5050–9.
[179] Koga Y, Matsuzaki A, Suminoe A, Hattori H, Hara T. Neutrophil-derived TNF-related apoptosis-inducing ligand (TRAIL): a novel mechanism of antitumor effect by neutrophils. Cancer Res 2004;64(3):1037–43.
[180] Markman JL, Porritt RA, Wakita D, Lane ME, Martinon D, Noval Rivas M, et al. Loss of testosterone impairs anti-tumor neutrophil function. Nat Commun 2020;11(1):1613.
[181] Sionov RV, Fainsod-Levi T, Zelter T, Polyansky L, Pham CT, Granot Z. Neutrophil cathepsin G and tumor cell RAGE facilitate neutrophil anti-tumor cytotoxicity. Oncoimmunology 2019;8(9):e1624129.
[182] Sionov RV, Lamagna C, Granot Z. Recognition of tumor nidogen-1 by neutrophil C-type lectin receptors. Biomedicines 2022;10(4):908.
[183] Gutiérrez-Fernández A, Fueyo A, Folgueras AR, Garabaya C, Pennington CJ, Pilgrim S, et al. Matrix metalloproteinase-8 functions as a metastasis suppressor through modulation of tumor cell adhesion and invasion. Cancer Res 2008;68(8):2755–63.
[184] Eruslanov EB, Bhojnagarwala PS, Quatromoni JG, Stephen TL, Ranganathan A, Deshpande C, et al. Tumor-associated neutrophils stimulate T cell responses in early-stage human lung cancer. J Clin Invest 2014;124(12):5466–80.
[185] Wu Q, He Z, Wang X, Zhang Q, Wei Q, Ma S, et al. Cascade enzymes within self-assembled hybrid nanogel mimicked neutrophil lysosomes for singlet oxygen elevated cancer therapy. Nat Commun 2019;10(1):240.
[186] Läubli H, Borsig L. Selectins promote tumor metastasis. Semin Cancer Biol 2010;20(3):169–77.
[187] Mi Z, Guo L, Liu P, Qi Y, Feng Z, Liu J, et al. "Trojan Horse" *salmonella* enabling tumor homing of silver nanoparticles via neutrophil infiltration for synergistic tumor therapy and enhanced biosafety. Nano Lett 2021;21 (1):414–23.
[188] Dong X, Chu D, Wang Z. Neutrophil-mediated delivery of nanotherapeutics across blood vessel barrier. Ther Deliv 2018;9(1):29–35.
[189] McDonald B, Spicer J, Giannais B, Fallavollita L, Brodt P, Ferri LE. Systemic inflammation increases cancer cell adhesion to hepatic sinusoids by neutrophil mediated mechanisms. Int J Cancer 2009;125(6):1298–305.
[190] Kang T, Zhu Q, Wei D, Feng J, Yao J, Jiang T, et al. Nanoparticles coated with neutrophil membranes can effectively treat cancer metastasis. ACS Nano 2017;11(2):1397–411.
[191] Li Y, Yang Y, Gan T, Zhou J, Hu F, Hao N, et al. Extracellular RNAs from lung cancer cells activate epithelial cells and induce neutrophil extracellular traps. Int J Oncol 2019;55(1):69–80.
[192] Lee J, Lee D, Lawler S, Kim Y. Role of neutrophil extracellular traps in regulation of lung cancer invasion and metastasis: Structural insights from a computational model. PLoS Comput Biol 2021;17(2):e1008257.
[193] Cools-Lartigue J, Spicer J, McDonald B, Gowing S, Chow S, Giannias B, et al. Neutrophil extracellular traps sequester circulating tumor cells and promote metastasis. J Clin Invest 2013;123(8):3446–58.
[194] Xue J, Zhao Z, Zhang L, Xue L, Shen S, Wen Y, et al. Neutrophil-mediated anticancer drug delivery for suppression of postoperative malignant glioma recurrence. Nat Nanotechnol 2017;12(7):692–700.
[195] De Larco JE, Wuertz BR, Furcht LT. The potential role of neutrophils in promoting the metastatic phenotype of tumors releasing interleukin-8. Clin Cancer Res 2004;10(15):4895–900.
[196] Chen HC, Lin HC, Liu CY, Wang CH, Hwang T, Huang TT, et al. Neutrophil elastase induces IL-8 synthesis by lung epithelial cells via the mitogen-activated protein kinase pathway. J Biomed Sci 2004;11(1):49–58.
[197] Sun T, Gao J, Han D, Shi H, Liu X. Fabrication and characterization of solid lipid nano-formulation of astraxanthin against DMBA-induced breast cancer via Nrf-2-Keap1 and NF-kB and mTOR/Maf-1/PTEN pathway. Drug Deliv 2019;26 (1):975–88.
[198] Zheng Z, Li YN, Jia S, Zhu M, Cao L, Tao M, et al. Lung mesenchymal stromal cells influenced by Th2 cytokines mobilize neutrophils and facilitate metastasis by producing complement C3. Nat Commun 2021;12(1):6202.
[199] Yin H, Lu H, Xiong Y, Ye L, Teng C, Cao X, et al. Tumor-associated neutrophil extracellular traps regulating nanocarrier-enhanced inhibition of malignant tumor growth and distant metastasis. ACS Appl Mater Interfaces 2021;13 (50):59683–94.
[200] Robichaud N, del Rincon SV, Huor B, Alain T, Petruccelli LA, Hearnden J, et al. Phosphorylation of eIF4E promotes EMT and metastasis via translational control of SNAIL and MMP-3. Oncogene 2015;34(16):2032–42.
[201] Furic L, Rong L, Larsson O, Koumakpayi IH, Yoshida K, Brueschke A, et al. eIF4E phosphorylation promotes tumorigenesis and is associated with prostate cancer progression. Proc Natl Acad Sci U S A 2010;107(32):14134–9.
[202] Graff JR, Konicek BW, Vincent TM, Lynch RL, Monteith D, Weir SN, et al. Therapeutic suppression of translation initiation factor eIF4E expression reduces tumor growth without toxicity. J Clin Invest 2007;117(9):2638–48.
[203] Robichaud N, Hsu BE, Istomine R, Alvarez F, Blagih J, Ma EH, et al. Translational control in the tumor microenvironment promotes lung metastasis: phosphorylation of eIF4E in neutrophils. Proc Natl Acad Sci USA 2018;115(10):E2202–9.
[204] Chu D, Dong X, Zhao Q, Gu J, Wang Z. Photosensitization priming of tumor microenvironments improves delivery of nanotherapeutics via neutrophil infiltration. Adv Mater 2017;29(27):1701021.
[205] Li P, Lu M, Shi J, Gong Z, Hua L, Li Q, et al. Lung mesenchymal cells elicit lipid storage in neutrophils that fuel breast cancer lung metastasis. Nat Immunol 2020;21(11):1444–55.
[206] Karpisheh V, Fakkari Afjadi J, Nabi Afjadi M, Haeri MS, Abdpoor Sough TS, Heydarzadeh Asl S, et al. Inhibition of HIF-1α/EP4 axis by hyaluronate-trimethyl chitosan-SPION nanoparticles markedly suppresses the growth and development of cancer cells. Int J Biol Macromol 2021;167:1006–19.
[207] Trotter TN, Shuptrine CW, Tsao LC, Marek RD, Acharya C, Wei JP, et al. IL26, a noncanonical mediator of DNA inflammatory stimulation, promotes TNBC engraftment and progression in association with neutrophils. Cancer Res 2020;80(15):3088–100.
[208] Masjedi A, Ahmadi A, Atyabi F, Farhadi S, Irandoust M, Khazaei-Poul Y, et al. Silencing of IL-6 and STAT3 by siRNA loaded hyaluronate-N, N, N-trimethyl chitosan nanoparticles potently reduces cancer cell progression. Int J Biol Macromol 2020;149:487–500.

[209] Jatal R, Mendes Saraiva S, Vázquez-Vázquez C, Lelievre E, Coqueret O, López-López R, et al. Sphingomyelin nanosystems decorated with TSP-1 derived peptide targeting senescent cells. Int J Pharm 2022;617:121618.
[210] Park J, Wysocki RW, Amoozgar Z, Maiorino L, Fein MR, Jorns J, et al. Cancer cells induce metastasis-supporting neutrophil extracellular DNA traps. Sci Transl Med 2016;8(361):361ra138.
[211] Demers M, Krause DS, Schatzberg D, Martinod K, Voorhees JR, Fuchs TA, et al. Cancers predispose neutrophils to release extracellular DNA traps that contribute to cancer-associated thrombosis. Proc Natl Acad Sci USA 2012;109 (32):13076–81.
[212] Qin J, Zhang Z, Fu Z, Ren H, Liu M, Qian M, et al. The UDP/P2y6 axis promotes lung metastasis of melanoma by remodeling the premetastatic niche. Cell Mol Immunol 2020;17(12):1269–71.
[213] Hayat SMG, Bianconi V, Pirro M, Jaafari MR, Hatamipour M, Sahebkar A. CD47: role in the immune system and application to cancer therapy. Cell Oncol 2020;43(1):19–30.
[214] Huh SJ, Liang S, Sharma A, Dong C, Robertson GP. Transiently entrapped circulating tumor cells interact with neutrophils to facilitate lung metastasis development. Cancer Res 2010;70(14):6071–82.
[215] Muro S, Dziubla T, Qiu W, Leferovich J, Cui X, Berk E, et al. Endothelial targeting of high-affinity multivalent polymer nanocarriers directed to intercellular adhesion molecule 1. J Pharmacol Exp Ther 2006;317 (3):1161–9.
[216] Yamamoto T, Kawada K, Itatani Y, Inamoto S, Okamura R, Iwamoto M, et al. Loss of SMAD4 promotes lung metastasis of colorectal cancer by accumulation of CCR1^{+} tumor-associated neutrophils through CCL15–CCR1 axis. Clin Cancer Res 2017;23(3):833–44.
[217] Ito Y, Onoda N, Kihara M, Miya A, Miyauchi A. Prognostic significance of neutrophil-to-lymphocyte ratio in differentiated thyroid carcinoma having distant metastasis: a comparison with thyroglobulin-doubling rate and tumor volume-doubling rate. In Vivo 2021;35(2):1125–32.
[218] Flower DR, North AC, Sansom CE. The lipocalin protein family: structural and sequence overview. Biochim Biophys Acta 2000;1482(1–2):9–24.
[219] Volpe V, Raia Z, Sanguigno L, Somma D, Mastrovito P, Moscato F, et al. NGAL controls the metastatic potential of anaplastic thyroid carcinoma cells. J Clin Endocrinol Metab 2013;98(1):228–35.
[220] Jeon HD, Han YH, Mun JG, Yoon DH, Lee YG, Kee JY, et al. Dehydroevodiamine inhibits lung metastasis by suppressing survival and metastatic abilities of colorectal cancer cells. Phytomedicine 2022;96:153809.
[221] Yang C, Sun W, Cui W, Li X, Yao J, Jia X, et al. Procoagulant role of neutrophil extracellular traps in patients with gastric cancer. Int J Clin Exp Pathol 2015;8 (11):14075–86.
[222] Bayda S, Adeel M, Tuccinardi T, Cordani M, Rizzolio F. The history of nanoscience and nanotechnology: from chemical-physical applications to nanomedicine. Molecules 2019;25(1):112.
[223] Wang L, Yan L, Liu J, Chen C, Zhao Y. Quantification of nanomaterial/nanomedicine trafficking *in vivo*. Anal Chem 2018;90 (1):589–614.
[224] Ali A, Ovais M, Zhou H, Rui Y, Chen C. Tailoring metal–organic frameworks-based nanozymes for bacterial theranostics. Biomaterials 2021;275:120951.
[225] Ovais M, Guo M, Chen C. Tailoring nanomaterials for targeting tumor-associated macrophages. Adv Mater 2019;31(19):e1808303.
[226] Zhou H, Qin F, Chen C. Designing hypoxia-responsive nanotheranostic agents for tumor imaging and therapy. Adv Healthc Mater 2021;10(5):e2001277.
[227] Ovais M, Nethi SK, Ullah S, Ahmad I, Mukherjee S, Chen C. Recent advances in the analysis of nanoparticle-protein coronas. Nanomedicine 2020;15 (10):1037–61.
[228] Chu D, Dong X, Shi X, Zhang C, Wang Z. Neutrophil-based drug delivery systems. Adv Mater 2018;30(22):e1706245.
[229] Ovais M, Mukherjee S, Pramanik A, Das D, Mukherjee A, Raza A, et al. Designing stimuli-responsive upconversion nanoparticles that exploit the tumor microenvironment. Adv Mater 2020;32(22):e2000055.
[230] Ni D, Lin J, Zhang N, Li S, Xue Y, Wang Z, et al. Combinational application of metal-organic frameworks-based nanozyme and nucleic acid delivery in cancer therapy. Wiley Interdiscip Rev Nanomed Nanobiotechnol 2022;14(3): e1773.
[231] Chen JW, Querol Sans M, Bogdanov Jr A, Weissleder R. Imaging of myeloperoxidase in mice by using novel amplifiable paramagnetic substrates. Radiology 2006;240(2):473–81.
[232] Tang L, Wang Z, Mu Q, Yu Z, Jacobson O, Li L, et al. Targeting neutrophils for enhanced cancer theranostics. Adv Mater 2020;32(33):e2002739.
[233] Chan LW, Anahtar MN, Ong TH, Hern KE, Kunz RR, Bhatia SN. Engineering synthetic breath biomarkers for respiratory disease. Nat Nanotechnol 2020;15(9):792–800.
[234] Zhang N, Francis KP, Prakash A, Ansaldi D. Enhanced detection of myeloperoxidase activity in deep tissues through luminescent excitation of near-infrared nanoparticles. Nat Med 2013;19(4):500–5.
[235] Liu SY, Yan AM, Guo WY, Fang YY, Dong QJ, Li RR, et al. Human neutrophil elastase activated fluorescent probe for pulmonary diseases based on fluorescence resonance energy transfer using CdSe/ZnS quantum dots. ACS Nano 2020;14(4):4244–54.
[236] Li YF, Chen C. Fate and toxicity of metallic and metal-containing nanoparticles for biomedical applications. Small 2011;7(21):2965–80.
[237] Li J, Liu J, Chen C. Remote control and modulation of cellular events by plasmonic gold nanoparticles: implications and opportunities for biomedical applications. ACS Nano 2017;11(3):2403–9.
[238] Chen Z, Mao R, Liu Y. Fullerenes for cancer diagnosis and therapy: preparation, biological and clinical perspectives. Curr Drug Metab 2012;13 (8):1035–45.
[239] Tang J, Zhang R, Guo M, Zhou H, Zhao Y, Liu Y, et al. Gd-metallofullerenol drug delivery system mediated macrophage polarization enhances the efficiency of chemotherapy. J Control Release 2020;320:293–303.
[240] Amulic B, Cazalet C, Hayes GL, Metzler KD, Zychlinsky A. Neutrophil function: from mechanisms to disease. Annu Rev Immunol 2012;30:459–89.
[241] Erpenbeck L, Schön MP. Neutrophil extracellular traps: protagonists of cancer progression? Oncogene 2017;36(18):2483–90.
[242] Powell DR, Huttenlocher A. Neutrophils in the tumor microenvironment. Trends Immunol 2016;37(1):41–52.
[243] Treffers LW, Hiemstra IH, Kuijpers TW, van den Berg TK, Matlung HL. Neutrophils in cancer. Immunol Rev 2016;273(1):312–28.
[244] De Santo C, Arscott R, Booth S, Karydis I, Jones M, Asher R, et al. Invariant NKT cells modulate the suppressive activity of IL-10-secreting neutrophils differentiated with serum amyloid A. Nat Immunol 2010;11(11):1039–46.
[245] Scapini P, Marini O, Tecchio C, Cassatella MA. Human neutrophils in the saga of cellular heterogeneity: insights and open questions. Immunol Rev 2016;273(1):48–60.
[246] Xu S, Wang C, Mao R, Liang X, Wang H, Lin Z, et al. Surface structure change properties: auto-soft bionic fibrous membrane in reducing postoperative adhesion. Bioact Mater 2021;12:16–29.
[247] Xu M, Zhang CY, Wu J, Zhou H, Bai R, Shen Z, et al. PEG-detachable polymeric micelles self-assembled from amphiphilic copolymers for tumor-acidity-triggered drug delivery and controlled release. ACS Appl Mater Interfaces 2019;11(6):5701–13.
[248] Zhou H, Hou X, Liu Y, Zhao T, Shang Q, Tang J, et al. Superstable magnetic nanoparticles in conjugation with near-infrared dye as a multimodal theranostic platform. ACS Appl Mater Interfaces 2016;8(7):4424–33.
[249] Wang Z, Liu Z, Mei J, Xu S, Liu Y. The next generation therapy for lung cancer: taking medicine by inhalation. Nanotechnology 2021;32(39):392002.
[250] Sun D, Gao W, Wu P, Liu J, Li S, Li S, et al. A one-pot-synthesized double-layered anticoagulant hydrogel tube. Chem Res Chin Univ 2021;37 (5):1085–91.
[251] Wu J, Zhang Z, Gu J, Zhou W, Liang X, Zhou G, et al. Mechanism of a long-term controlled drug release system based on simple blended electrospun fibers. J Control Release 2020;320:337–46.
[252] Xia Q, Zhang N, Li J, Wang H, Wang C, Zhang Z, et al. Dual-functional esophageal stent coating composed of paclitaxel-loaded electrospun membrane and protective film. J Biomed Nanotechnol 2019;15(10): 2108–20.
[253] Liu J, Wang P, Zhang X, Wang L, Wang D, Gu Z, et al. Rapid degradation and high renal clearance of Cu_3BiS_3 nanodots for efficient cancer diagnosis and photothermal therapy *in vivo*. ACS Nano 2016;10(4):4587–98.
[254] Gao S, Jiang S, Qi J, Wu T, Wang W, Liu Z, et al. Neither fluorocarbons nor silicones: hydrocarbon-based water-borne healable supramolecular elastomer with unprecedent dual resistance to water and organic solvents. CCS Chemistry. In press.
[255] Zhang C, Bai X, Chen S, Dedkova LM, Hecht SM. Local conformational constraint of firefly luciferase can affect the energy of bioluminescence and enzyme stability. CCS Chemistry. In press.
[256] Hu K, Zhou H, Liu Y, Liu Z, Liu J, Tang J, et al. Hyaluronic acid functional amphipathic and redox-responsive polymer particles for the co-delivery of doxorubicin and cyclopamine to eradicate breast cancer cells and cancer stem cells. Nanoscale 2015;7(18):8607–18.
[257] Wang L, Liu Y, Li W, Jiang X, Ji Y, Wu X, et al. Selective targeting of gold nanorods at the mitochondria of cancer cells: implications for cancer therapy. Nano Lett 2011;11(2):772–80.
[258] Saleem J, Wang L, Chen C. Carbon-based nanomaterials for cancer therapy via targeting tumor microenvironment. Adv Healthc Mater 2018;7(20): e1800525.
[259] Jin J, Guo M, Liu J, Liu J, Zhou H, Li J, et al. Graphdiyne nanosheet-based drug delivery platform for photothermal/chemotherapy combination treatment of cancer. ACS Appl Mater Interfaces 2018;10(10):8436–42.
[260] Yan X, Lin W, Liu H, Pu W, Li J, Wu P, et al. Wavelength-tunable, long lifetime, and biocompatible luminescent nanoparticles based on a vitamin E-derived material for inflammation and tumor imaging. Small 2021;17(25):e2100045.
[261] Pulli B, Wojtkiewicz G, Iwamoto Y, Ali M, Zeller MW, Bure L, et al. Molecular MR imaging of myeloperoxidase distinguishes steatosis from steatohepatitis in nonalcoholic fatty liver disease. Radiology 2017;284(2): 390–400.